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PREFATORY NOTE

THE EDITORS have received so many enquiries concerning the typography and general format of the *Journal* that they take this opportunity of acknowledging their indebtedness to Mr. Carl Purington Rollins, Printer to Yale University, who selected the type and designed the *Journal* page. The face employed is Monotype Century Schoolbook No. 420, 10 point on 12 point for ordinary text, and 8 point on 9 point for small print, footnotes and bibliography. After the May issue of 1938 a special font of Century Schoolbook No. 420 type with long descenders was adopted. The type selected has enabled the *Journal* to give a readable five-inch line with an unusually high word count per page (550 to 580) without diminishing legibility or violating esthetic typography. The new front cover for the second volume was also designed by Mr. Rollins.

The *Journal* has somewhat modified its policies with regard to its free allowance for illustrations, a new statement of these matters being found on the inside of the front cover. Attention is also directed to the Style Book which is included at the end of the present number. This contains suggestions for the preparation of manuscripts for the *Journal*, and also an extensive alphabet of journals with official *World list* abbreviations opposite. These abbreviations represent the only consistent system so far available for the world literature of medicine and science. Separate copies of the Style Book are offered gratis to contributors and additional copies are supplied by the publishers or editors at a charge of 25 cents.

THE EDITORS wish also to express their warm appreciation to Mr. Thomas, the publisher, and to the George Banta Publishing Company, Menasha, Wisconsin, for their lively interest in everything pertaining to the welfare of the *Journal*. To the contributors and subscribers who have helped in the successful launching of the *Journal* THE EDITORS also extend their thanks.

ABERRANT GANGLION CELLS AS A SOURCE OF INTACT FIBERS IN SEVERED DORSAL ROOTS

DONALD DUNCAN AND E S CROCKER

Department of Anatomy, University of Texas, Galveston

INTRODUCTION

IT IS BELIEVED that Toennies' (1938) confirmation of earlier investigations demonstrating the passage of nerve impulses centrifugally over the dorsal roots will stimulate further investigation of the histological aspects of the problem For this reason, and because an explanation has been found for some of the previously published results, a brief report of a long series of experiments on the subject conducted in this laboratory is presented.

Past observation of the presence or absence of histologically efferent dorsal root fibers in mammals may be divided into three groups: (i) efferent fibers are numerous, Kuré and associates (see Kuré and Kajiyama, 1936), and Barron and Matthews (1935); (ii) efferent fibers are present but in small variable numbers, Kahr and Sheehan (1933), Okelberry (1935), and Young and Zuckerman (1937); (iii) efferent fibers are probably absent, Hinsey (1934). Each conclusion has been published repeatedly in the past and the literature on the subject may be found in Hinsey's thorough review. Papers of the first category have been convincingly explained and refuted by Storey, Hinsey *et al.* (1936) and by Young and Zuckerman. But the results of those finding a few intact fibers in the central stump after section have either been accepted as proof of intraspinal origin or explained away as due to some error in method. Neither explanation is adequate if the observations presented in this paper are correct; namely, intact fibers are found in varying small numbers in the central stumps of severed dorsal roots because they arise from aberrant ganglion cells that lie in the root between the point of section and the spinal cord.

Aberrant ganglia are mentioned in all the larger current text-books of gross anatomy and occasional original papers on the subject have appeared for many years. However, no mention of the aberrant cells as a source of intact fibers in the dorsal roots following section has been found. The one possible exception to this statement is a line by Hinsey to the effect that one preparation was discarded because it contained ganglion cells, but no further mention of them was made in his paper.

MATERIALS AND METHODS

Experimental section of lumbo-sacral dorsal roots was performed on 35 cats The operations varied from section of a single rootlet near the spinal cord to extradural section of the second lumbar to the second sacral dorsal roots of one side The operations included 9 attempts to isolate single rootlets by section of the adjacent ones, above and below. In all cases the central stump was fixed in 1 per cent osmic acid and the distal stump, the ganglion, and the trunk of the affected root, as well as the adjacent uninjured roots were fixed and stained according to Marchi's method All material was examined in carefully teased preparations Subsequently 8 of the largest lumbar roots from 2 cats were cut in

serial longitudinal sections, stained with thionin and each section was carefully examined for nerve cells. Lower lumbar roots from 2 normal dogs and 2 dogs subjected to dorsal root section were similarly examined for nerve cells. In both of the operated dogs lower lumbar roots were sectioned extradurally. In one case the animal lived 21 days after section and in the other 30 days. Ten lumbar roots from 3 normal dogs were stained in bulk with carmine and then teased and examined for the presence of extra-ganglionic nerve cells.

RESULTS

It is considered unnecessary to report the results from the 35 cats in detail. With a minor exception they were in complete agreement with Hinsey's previous results and conclusions for the same species. The slight exception noted to Hinsey's results was the persistent finding of occasional normal fibers in the central stump and degenerating fibers in the distal stump.

Table 1. Incidence of aberrant cells in lumbar dorsal roots

Animal	Root examined	Extradural cells	Intradural cells
Cat 9–38	R. & L. 7L		0
Cat 10–38	R. & L. 5, 6, 7L		10
Dog 1–38	L5L	16	0
	L6L	10	0
	L7L	6	0
Dog 2–38	R7L	41	
	L7L	134	97
Dog 4–38	R6L*	39	
	R7L*	208	
Dog 5–38	L6L*	12	Numerous in
	R7L	111	teased roots of
	L1S*	227	L6L & L1S

* These roots were divided 5 mm. from their ganglia 21 days (Dog 4) and 30 days (Dog 5) before sacrifice.

Such fibers were always few in number and were found in about one half of all preparations. It was not until after 25 cats had been operated upon and examined that a satisfactory explanation for the occurrence of these occasional apparently efferent fibers was discovered. At this time ganglion cells with attached intact fibers were observed in the central stump of a dorsal root that had been cut intradurally about halfway between the ganglion and the spinal cord (Fig. 1 and 2). The results of the additional work performed to extend and confirm the observation are described in the succeeding paragraphs.

The numerical results of a search for nerve cells in the dorsal roots are recorded in Table 1. As indicated in the table such cells are rare in the cat and much more numerous in the dog. A similar difference was recorded by Peters (1935) for aberrant cells in the sensory root of the trigeminal nerve. In addition to cells recorded in the table, aberrant cells were found in the

teased preparations of the central ends of the cut dorsal roots in 4 instances out of 30 cat specimens examined for this feature. Since the entire root could not be examined in these cases, and since an occasional normal fiber was found in 15 of these preparations, it is believed that serial sections of the entire roots would have revealed enough additional cells to account for the intact fibers. An undetermined number of aberrant cells were also seen in each of 10 lumbar dorsal roots from 3 dogs. These roots had been stained in bulk with carmine and teased out in glycerine. It was considered that a sufficient number of specimens from the dog had thus been examined to establish the constant occurrence of aberrant dorsal root ganglion cells in this species.

Conditions in the dog are somewhat different from those in the cat, not only in regard to the number but also the location of the cells. In the dog the extradural portions of the lumbo-sacral roots are long as compared to the more common arrangement in man, rat, rabbit, and cat. This meningeal relation in the dog has been illustrated by Papez (1929, p. 137), but is considered of sufficient importance to investigators to be noted again, (Fig. 3). Most of the aberrant cells are found in the long extradural portion of the root, but intradural cells are much more common in the dog than in the cat. The largest collections of aberrant cells occur just outside the dura (Fig. 4). Single cells are fairly numerous throughout the entire extent of each root examined.

Survival of these cells and their processes following dorsal root section was easily demonstrated in the dogs following operation. The cells shown in Fig. 4 are from a root divided peripheral to these cells. Teased osmium treated preparations of the intradural portion of this root contained numerous normal fibers of all calibers and additional ganglion cells as well. Many of the aberrant cells in the divided roots exhibited typical retrograde changes (Fig. 5). According to Hinsey, Krupp, and Lhamon (1937) this indicates that the peripheral processes, rather than the central, were cut.

DISCUSSION

Although Barron and Matthews stated that as many as 32 per cent of the fibers of the dorsal roots of the cat failed to degenerate following separation from the ganglia, careful repetition of their procedures failed to establish anything contrary to the previous conclusions of Hinsey which have been subsequently restated by Storey, Corbin, and Hinsey. The occasional normal fibers found in the cat are so few in number and so readily accounted for by the presence of a like incidence of aberrant cells, that they must be considered as a minor exception to the complete degeneration that takes place in the central end of the severed dorsal root in this animal.

In the dog, nerve cells in the roots are sufficient in number to be a considerable source of confusion. Their incidence is great enough to account for Okelberry's (1935) convincing demonstration of intact fibers in the severed dorsal roots of this animal. Okelberry's results have been dismissed by several writers on the basis of the difficulty of cutting the roots extradurally with-

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All figures are unretouched photographs taken under the magnification indicated for each figure.

FIG. 1. An aberrant dorsal root ganglion cell found in the left seventh lumbar root of cat 10-38. ×460.

FIG. 2. Aberrant ganglion cells in a teased preparation of the central end of a lumbar dorsal root which had been divided 21 days before fixation, from cat 4-37. ×135.

(Continued next page)

out involvement of the ganglion. They point to the lesser number of fibers found after intradural section as proof of this contention. Such criticism of Okelberry's results is entirely unjustifiable because of the ease of extradural section in the dog. Even if the lumbar roots were sectioned as much as an inch away from the ganglion in this animal, fairly numerous normal fibers would usually be found in the intradural portions of the roots involved. It is believed that any careful repetition of Okelberry's work would completely substantiate his findings, but that such results are to be accounted for almost if not entirely on the basis of extra-ganglionic cells in the roots and not by assuming that the remaining intact fibers arise from the cord. The results obtained by Young and Zuckerman on the monkey cannot be due entirely to the presence of aberrant cells, but their claims of an appreciable number of fine myelinated efferent fibers in the monkey should be viewed with caution because of the great difficulty of distinguishing normal fibers from unfragmented segments of fibers undergoing degeneration. This is especially true of the myelinated fibers of fine caliber studied in cross sections.

As mentioned in the introduction, nerve cells in the dorsal roots as aberrant ganglia have been described repeatedly in the past and statements concerning them may be found in the current editions of texts of Cunningham, Gray, Morris, and Piersol. The occurrence of these cells as isolated and scattered elements has been mentioned most recently by Tarlov (1937). The earlier papers on aberrant cells are cited by Tarlov and by Peters.

SUMMARY AND CONCLUSIONS

Experimental section of the lumbo-sacral dorsal roots was performed on 35 cats and 2 dogs. After an interval for degeneration occasional normal myelinated fibers were seen in the central ends of the severed roots from 15 of the cats and fairly numerous normal fibers were seen in the central stumps from both dogs.

Aberrant dorsal root ganglion cells were found in the central ends of the cut dorsal roots from 4 of the cats and both dogs. The qualitative presence of aberrant cells in each of 10 normal roots from 3 dogs was established.

Serial sections of lumbo-sacral dorsal roots from 2 normal cats and 4 dogs, 2 normals and 2 operated, were examined and the number of aberrant cells was determined.

In the cat the number of aberrant cells is small, some roots containing

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FIG. 3. A dissection of the lower lumbar and sacral roots of a young adult police dog; Close examination of the figure will show that approximately one half of the length of any of these roots is extradural. ×$\frac{1}{2}$.

FIG. 4. A section from dog 5–38 showing a normal ventral root, V. R., and portions of a severed dorsal root, D. R., at the point where these roots pass through the dura. A small collection of ganglion cells is shown in the extradural portion of the dorsal root. These cells are at least an inch central to the main ganglion. ×45. Insert "a" shows the single intradural cell indicated by the arrow on the main figure at a higher magnification. ×135.

FIG. 5. Aberrant cells from dog 4–38 showing eccentric nuclei and chromatolysis in a dorsal root severed 21 days before fixation. ×460.

none at all and not more than 6 were seen in any one root. A total of 10 were seen in the 8 roots examined in serial section. In the dog the cells are much more numerous, and no root was examined that did not contain some of them. Most of these cells are found in the long extradural portion of the root, but they are also found in smaller numbers intradurally. In the 10 roots completely examined, the number of cells in the extradural portion varied from 6 to 227.

These cells and their processes survive section of the roots and are believed to account for the so-called efferent fibers that have been demonstrated in otherwise adequately controlled experiments.

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THE LOCALIZATION IN THE BRAIN STEM OF THE OESTROUS RESPONSES OF THE FEMALE GUINEA PIG[1]

EDWARD W. DEMPSEY[2] AND DAVID McK. RIOCH

From the Department of Anatomy, Harvard Medical School, Boston

IT HAS BEEN shown previously that a well-defined pattern of reflex behavior of the female guinea pig begins suddenly at oestrus and persists for a definite period (Young, Myers and Dempsey, 1935). This reflex pattern is elicited by rubbing or touching the hair and skin on the dorsal lumbar and perineal regions, or by the sexual behavior of the male. The pattern is characterized by opisthotonus, an elevation of the pelvis, a dilatation of the vulvar regions, and it is frequently accompanied by a guttural, purring vocalization. The close relation of this behavior to the endocrine events of the reproductive cycle has been demonstrated by its synchronization with the pre-ovulatory changes in the Graafian follicles (Myers, Young and Dempsey, 1936), and by the ease with which it can be produced experimentally by appropriate hormone injections (Dempsey, Hertz and Young, 1936).

The desirability of determining the neural mechanism responsible for the control of oestrous behavior immediately became apparent after the establishment of the facts mentioned above. Bard and Rioch (1937) and Brooks (1937) have demonstrated, for the cat and rabbit respectively, that oestrous behavior can occur in animals which have been completely deprived of the neocortex. It seemed desirable, therefore, to learn more about the nervous elements involved in sexual behavior. The present paper represents a series of experiments which have been designed to determine the approximate localization in the brain stem and spinal cord of these reflex mechanisms.

MATERIALS AND METHODS

Brain lesions of varying extent were produced surgically in ovariectomized guinea pigs Following the operative procedures, suitably timed injections of oestrin and progesterone were made, as it has been shown previously that such hormonal treatment in spayed females is invariably followed by oestrous behavior (Dempsey, Hertz and Young, 1936) Whenever possible, chronically operated animals were maintained for considerable periods of time and tested for sexual receptivity after repeated injections of the hormones However, many of the individuals in which large lesions had been produced, particularly lesions involving bilateral destruction of the motor cortex, refused to eat after the operation and became moribund within a few days With these animals it was necessary to resort to subacute procedures, and the injections and tests for sexual behavior were carried out immediately after the operation, while the general condition of the animal was still good

The tissue to be removed was either cut out under anesthesia with a sharp spatula or sucked out through a fine glass tube attached to a suction pump

At the sacrifice of each animal, the brain was removed and fixed in 10

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per cent formalin. Gross dissection was employed in the case of several specimens to determine the extent of the lesion, while in other specimens serial sections 35 μ in thickness were prepared and stained in thionin. In addition to the lesions produced in ovariectomized guinea pigs, transection of the brain stem at three different levels was performed in an acute experiment in an oestrous female cat. This experiment, which was undertaken in collaboration with Dr. Robert S. Morison of the Department of Physiology, has provided information similar to that arising from the guinea pig experiments, and is therefore included in this report.

<center>EXPERIMENTAL</center>

Cortical and striatal lesions

Chronic survival experiments. At the beginning, attempts were made to prepare chronic ovariectomized, decorticate guinea pigs by a three-stage
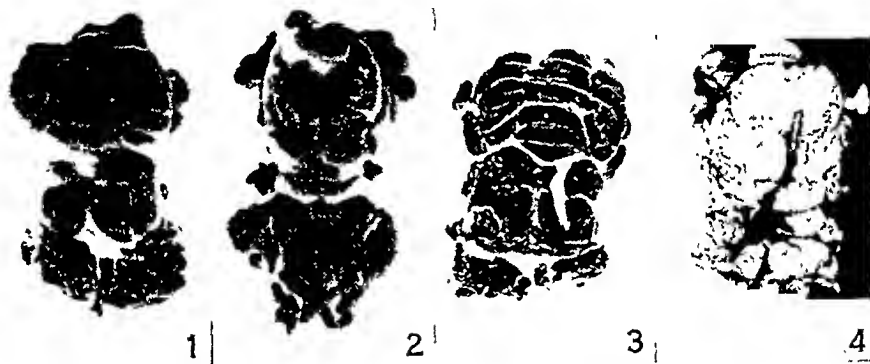
FIG. 1 and 2. Dorsal and ventral views of the brain of a decorticate guinea pig which came into heat following injections of oestrin and progesterone. Sections through the brain of this animal are shown in Fig. 6, 7 and 8.

FIG. 3 and 4. Dorsal and ventral views of the brain of a guinea pig in which acute decortication was performed. Oestrous responses were obtained after the first transection, not after the second.

operative procedure. It rapidly became evident, however, that even hemidecortication is often followed by death because of refusal of the animal to eat. Forced feeding was undertaken postoperatively with only slight prolongation of life, since it was impossible to supply the large quantity of food which normally is consumed by these rodents. Nevertheless, 9 animals survived hemidecortication for varying periods of time. These individuals were injected with 100 I. U. oestrin (Progynon-B, Schering)[2] daily for two days, and 0.1 I. U. progesterone (Proluton, Schering) was administered 48 hours after the first injection of oestrin. Six of the 9 females came into full behavioral

oestrus within the usual latent period of 3 to 7 hours after the progesterone injection. Completely normal reflex responses were obtained, and no difference was observed on the two sides of the animals with regard to the threshold of stimulation necessary to elicit the response.

Acute terminal experiments. Since the preparation of chronic, surviving decorticate animals was not feasible, an attempt was made to induce oestrous behavior in animals in which the cortex was removed acutely (Fig. 1 and 2). Four animals were injected with 250 I. U. oestrin and 24–36 hours later the cortex and other portions of the forebrain were bilaterally ablated under ether anesthesia. Forty-eight hours after the injection of oestrin, 0.1 I. U. progesterone was administered and oestrous responses were demonstrable in two of the four preparations 4 and 5 hours respectively after the progesterone injection. The responses in these animals were unmistakable, and were accompanied in one instance by the purring vocalization which is often elicited with the oestrous response in normal animals. Although completely normal in all respects, the responses elicited from these two animals were easily fatigued. Only two or three responses could be evoked in quick succession, after which a period of 10 to 15 minutes' rest was necessary before the response again appeared on stimulation, although in normal animals 20 or 30 responses may be obtained easily in rapid succession.

Histological examination of the brains of these animals showed that the neocortex and hippocampus had been completely removed in both instances. In both animals considerable damage had also been done to the caudate-putamen, only a few of the most medially located cells remaining in one case (Fig. 6, 7 and 8), while in the other animal the medial half of the complex remained on the right side and had been completely removed on the left. The olfactory stalks had been completely severed in one animal, while in the other they remained intact.

Medial and basilar forebrain lesions

Chronic survival experiments. Lesions were produced by means of a small sucker in the septal regions in 9 animals, involving the septal nuclei, the anterior commissure, the fornix and the medial half of the tuberculum olfactorium (Fig. 9, 10 and 11). After recovery from the operation, these animals were injected with oestrin and progesterone, and sexual behavior occurred in 8 cases. No difference in the stimulation threshold of the reflex could be noted on the two sides of any animal, although examination of the brains of these individuals showed that the lesion was not symmetrical in all cases, and in some instances structures on only one side had been destroyed.

Similar but more extensive lesions involving the pre-optic and anterior hypothalamic areas were also made, but in no case was oestrous behavior observed when oestrin and progesterone were injected. However, these animals rapidly became cachectic and showed such disturbances in water metabolism and heat regulation that their failure to show oestrous responses could well be attributed to general systemic effects.

Acute terminal experiments. Sexual behavior was observed in an acute experiment in which the anterior hypothalamus had been removed. An ovariectomized guinea pig was injected with oestrin and progesterone, and immediately following the injection of progesterone the brain stem was transsected at a level just anterior to the mammillary bodies (Fig. 3 and 4). Seven hours later, unmistakable oestrous reflexes were observed when the lumbar and perineal regions of the animal were stimulated. A second cut was then made at a level just anterior to the superior colliculus and just behind the mammillary bodies, after which no further responses referable to oestrous behavior were obtained (Fig. 5).
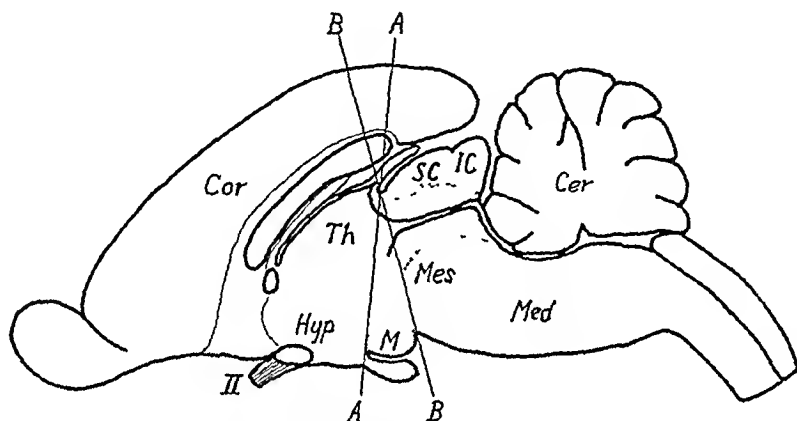
FIG. 5. Diagram of medial sagittal section through brain of guinea pig. Cor—cortex, Th—thalamus, Hyp—hypothalamus, M—mammillary body, SC—superior colliculus, IC—inferior colliculus. Mes—mesencephalon, Med—medulla, Cer—cerebellum, II—optic nerve. Oestrous responses were obtained after transection along plane AA but were abolished by transection along plane BB. Lesions in the optic tectum shown by the stippled area also abolish oestrous responses

Results similar to those described in the preceding paragraph were also obtained in an acute experiment in an oestrous cat. On the third day following an intramuscular injection of 10,000 I. U. oestrin, well-marked oestrous responses were observed. These responses were elicited by manual stimulation of the perineal regions, and consisted of growling, crouching with the forefeet and active treading with the hindlegs. Upon cessation of stimulation, the cat lay on her side and repeatedly rubbed her cheek and occiput against the floor.

Under ether anesthesia a tracheal cannula was inserted, the skull opened, and decortication performed by a sloping transection of the brain stem from a point 2 to 3 mm. rostral to the superior colliculi to a point just rostral to the optic chiasm. All brain tissue anterior to the cut was removed, the hemorrhage controlled, and the animal placed on the floor to recover from the anesthesia. An hour later, manual stimulation of the external genitalia was followed by shortening of the long back muscles and extension of the hindlegs. When supported on all four feet, stimulation of the genitalia caused an elevation and rotation of the pelvis, flexion of the front legs, and extension of the

hind legs with the result that the cat assumed a crouching posture with the hind legs extended. Insertion of a glass rod into the vagina caused a marked hyperpnoea characterized by intermittent prolonged exhalation, crouching on the fore quarters, together with extension and treading movement of the hind legs. A *second* transection of the brain stem was then made from the same level dorsally to a point just rostral to the mammillary bodies. Stimulation of the external genitalia and insertion of a glass rod into the vagina were followed by reactions identical to, but weaker and less easily elicited than, those described in the preceding paragraph. A *third* transection was made through the brain stem at the intercollicular level, and was followed by the development of good decerebrate rigidity. Stimulation of the genitalia, both manually and by glass rod inserted *per vaginam*, was followed by no reactions comparable to those described above.

Mesencephalic lesions

Complete transections. Completely negative results, similar to those described in the preceding paragraphs, were obtained in several experiments in which the mesencephalon was totally transected at the intercollicular level. Seven guinea pigs were injected with oestrin, and progesterone was administered 48 hours later. Immediately following the injection of progesterone, 4 animals were decerebrated at the intercollicular level by the Sherrington trephine method and 3 by the anemic method developed for the cat by Pollock and Davis (1924). Likewise, similar transections were produced in 3 oestrous cats. In all cases marked decerebrate rigidity developed immediately after the operation, but in no case was any response obtained which even remotely resembled oestrous behavior.

Partial transections. Experiments were next undertaken which were designed to test the assumption that the failure of the decerebrate animals to show oestrous responses was due to specific interference with the reflex mechanisms by means of which these responses are mediated rather than to general systemic effects. The mesencephalon was partially transected by means of the sucker. The cut extended completely across both inferior colliculi to depths varying from the dorsal margin of the central gray around the aqueduct of Sylvius (Fig. 12, 13 and 14) to the aqueduct itself. These lesions were well tolerated by the 3 guinea pigs in which they were produced. Repeated injections of oestrin and progesterone were made weekly in these animals for a period of 3 months, but in no instance was oestrous behavior observed. In addition to the loss of oestrous responses, all three of these guinea pigs showed a sensory tactile deficiency. Stimulation of the animal by touching or rubbing the hair on the back or flanks was followed by no evading reaction whatever, although normal guinea pigs react promptly to such stimulation by jumping and running away. This deficiency would seem to be confined solely to the exteroceptors in the hair follicles, since the stronger stimulation of rubbing the skin of the animals was followed promptly by evading responses. Likewise, the reactions to nociceptive stimulation, *e.g.*, pinching the skin or pulling the hair, appeared to be normal.
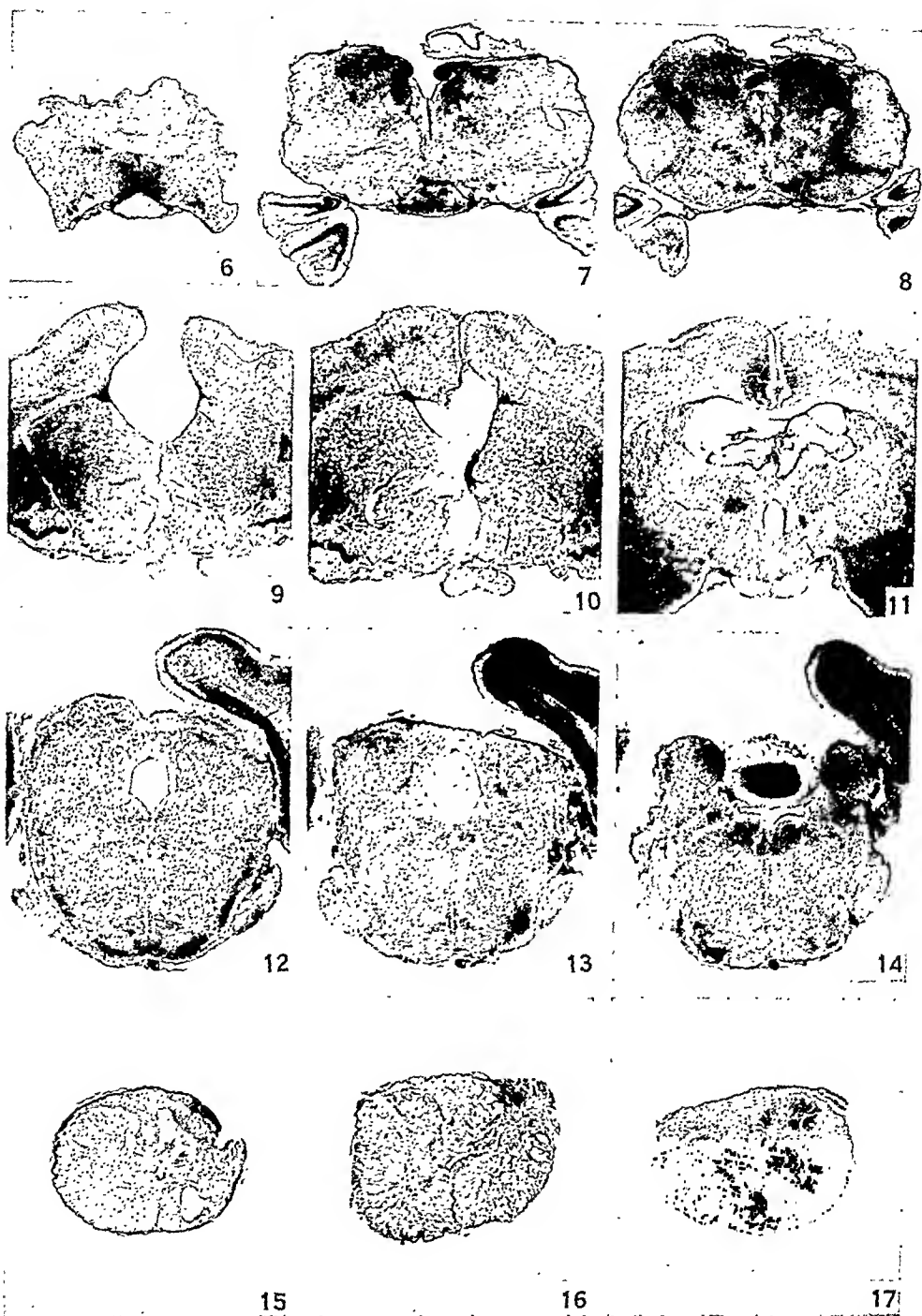
FIGS. 6–17. (*For legends see opposite page.*)

Lesions similar to those mentioned above, but involving the inferior colliculus on only one side, have been produced in 4 animals. These individuals also show a tactile deficiency which is sharply limited to the side of the body opposite the lesion. Likewise, exactly similar symptoms were observed in 2 animals in which one superior colliculus was destroyed in the same fashion. Injections of oestrin and progesterone have been made repeatedly into these animals and in two instances oestrous responses were obtained. These responses, however, were weak and required such strong stimulation that the laterality of the reflexogenous zone was not determinable.

Spinal cord lesions

Complete transections. Transections of the spinal cord at levels ranging from L2 to T5 were made in a series of 8 spayed guinea pigs. After recovery from the operation the spinal reflexes were tested repeatedly, both before and after the injection of oestrin and progesterone. No change could be found in the spinal reflexes which was correlated with the endocrine condition of the animal, and in no case was any reflex obtained which was at all suggestive of the responses which normally are obtained at oestrus. Three animals, however, were observed to utter a purring vocalization which frequently accompanies oestrus when the skin and hair on the back were stimulated above the level of the transection.

Hemisections. In 3 spayed animals an attempt was made to hemisect the spinal cord laterally at the C2 level. After recovery from the operation, these animals were injected with oestrin and progesterone and tested for sexual behavior. Completely normal responses were obtained repeatedly from all three; the reflexes involving the hind-legs and perineum were bilaterally expressed. These responses, however, could be obtained only by stimulating the hair on the side opposite the lesion, while stimulation of the side ipsilateral to the lesion evoked no response whatever. These animals were also tested for the tactile deficiency mentioned above during the intervals between the

Fig. 6, 7 and 8 Transverse sections through the optic chiasm, mammillary bodies and posterior commissure of the decorticate guinea pig shown in Fig. 1 and 2. Thionin. ×7

Fig 9, 10 and 11 Transverse sections through the region of the optic chiasm of the brain of a guinea pig in which the septal regions had been removed and the pre-optic area injured Normal oestrous responses were obtained from this animal. Thionin. ×7.

Fig 12, 13 and 14. Transverse sections through the region of the inferior colliculus of the brain of a guinea pig in which the mesencephalic tectum was partially transected at the level of the inferior colliculi No oestrous responses were obtained from this animal, and a sensory tactile deficiency was also present (see text). Thionin. ×7.

Fig. 15 Transverse section through the C2 segment of the spinal cord of a guinea pig after hemisection. Bilaterally expressed oestrous responses were obtained, but only after stimulation of the normal side Hematoxylin and eosin. ×8.

Fig. 16. Transverse section through the C2 segment of the spinal cord of a guinea pig after destruction of the posterior quadrant. The behavior of the animal was identical with that of the animal shown in Fig. 15 Hematoxylin and eosin. ×8

Fig 17 Transverse section through the C2 segment of the spinal cord of a guinea pig after destruction of the anterior quadrant. The oestrous behavior of this animal was completely normal. Hematoxylin and eosin ×8.

oestrous periods induced by the hormone injections. All three individuals showed the deficiency on the side ipsilateral to the lesion, while normal evading responses were obtained from stimulation of the hair on the contralateral side.

Histological examination of sections from the C2 segment showed that complete hemisection had been produced in only one of the three cases. The lesion was practically confined to the posterior quadrant in one case, while in the third case considerable damage was done to the anterior quadrant in addition to complete destruction of the posterior quadrant (Fig. 15).

Posterior quadrant section. The posterior quadrant of the cord was sectioned at the C2 level in 2 animals. After the operation, both failed to show evading responses when the hair was touched on the ipsilateral side, while stimulation on the side contralateral to the lesion was followed by these reactions. One animal failed to make a good recovery from the operation and died a few days later, but injection of the remaining animal with oestrin and progesterone was followed by oestrous responses which were bilaterally expressed but which could be elicited only by stimulating the side contralateral to the lesion. Examination of the C2 segment of the cord showed that the lesion had successfully interrupted the posterior column, Lissaur's tract and other regions in the posterior quadrant (Fig. 16).

Anterior quadrant section. Lesions involving one anterior quadrant of the cord also were made at the C2 level in 2 animals (Fig. 17). After recovery, evading responses were obtained from either side of the animals, and, after injection with oestrin and progesterone, completely normal bilaterally expressed oestrous responses could be obtained from stimulation of either side of the animal.

DISCUSSION

The experiments just described constitute proof that normal sexual responses can occur in the complete absence of the neocortex and of certain other portions of the forebrain in the guinea pig. Likewise, it has been shown elsewhere that there is in the electro-encephalogram of guinea pigs no change which can be correlated with the state of sexual receptivity (Dempsey, unpublished data). These experiments are in complete agreement with those of Bard and Rioch (1937), Bard (1936), and Brooks (1937), and show that normal oestrous responses can occur in animals which have been deprived of neocortex.

In addition, it has been demonstrated that lesions involving the septal regions and other medially located structures do not interfere with the oestrous responses. Likewise, oestrous responses have been obtained in acute experiments after removal of all tissue in front of a plane extending from the anterior limits of the superior colliculi to the anterior margin of the mammillary bodies. However, removal of the mammillary bodies by a second transection of the brain stem and transections at the intercollicular level abolish the oestrous responses. These experiments indicate that a fundamental part of the mechanism which controls the mating reactions is located caudal to the

anterior margin of the mammillary bodies and rostral to the intercollicular level. The loss of the sexual reactions after destruction of the colliculi can best be interpreted as due to an interruption of the afferent pathways associated with these responses (see below). This constitutes further evidence that the caudal limit of the region which controls the mating responses lies at a level anterior to the colliculi.

A normal female guinea pig, when not in heat, shows evading reactions to the same stimulus which evokes sexual behavior when the animal is in heat. Both the evading reactions and the sexual responses are lost with the same lesions of the colliculi and of the cord, whereas both are bilaterally preserved in surviving hemidecorticate animals and animals with asymmetrical septal lesions. It therefore seems likely that the same exteroceptors and central ascending pathways are involved in both of these patterns of behavior. The fact that unilateral lesions of the colliculi abolish the evading reactions from the contralateral side, but cause a general depression of the oestrous behavior, does not necessarily constitute evidence against the above hypothesis, but may only indicate the greater vulnerability of the oestrous mechanism.

The unilateral lesions in the spinal cord and mesencephalon which have been described in preceding sections permit an approximate localization of the tracts involved in the mediation of sexual responses in the guinea pig. Since unilateral destruction of the cord or of the posterior quadrant reduces the reflexogenous zone to the side of the body contralateral to the lesion, it would seem likely that the afferent supply of the reflex responses is associated with the posterior columns and remains uncrossed in the cord. Likewise, since there is a contralateral deficiency involving the exteroceptors which set off the evading reactions and the oestrous responses after destruction of one inferior or superior colliculus, it would seem that this tract decussates somewhere below the level of the colliculi and that it runs through the tectum mesencephali. Less is known of the efferent side of the reflexes. However, since unilateral section of the cord is followed by bilaterally expressed reflexes, the efferent supply of these reflexes must cross in the cord at levels caudal to C2.

In conclusion it may be pointed out that the present experiments offer further circumstantial evidence in favor of the concept that oestrous behavior results from the action of the sex hormones on the central nervous system, and that the site of this action lies in the brain (Sherrington, 1906). Absence of changes in the electro-encephalogram and in the spinal cord reflexes, together with the evidence that long afferent paths which decussate in the hind brain are involved, suggests that the site of action of the hormones concerned (oestrin, progesterone) is on the center which has been here localized at a level between the anterior margin of the mammillary bodies and the intercollicular plane.

CONCLUSIONS

Typical oestrous responses have been obtained from ovariectomized guinea pigs after suitable hormone injections following removal of neocortex, caudate-putamen, hippocampus, septal nuclei and other portions of the forebrain.

Likewise, oestrous responses have been observed in the guinea pig and the cat after transection of the brain stem at a level just anterior to the mammillary bodies. Partial or complete transection of the brain stem at the level of the inferior colliculi abolishes sexual behavior, and it is therefore thought that the anterior limit of the neural mechanism which controls sexual behavior lies between the intercollicular level and the anterior limit of the mammillary bodies.

Unilateral lesions in the posterior quadrant of the cord are followed by completely normal and bilaterally equal sexual responses, but these responses can be evoked only by stimulation of the animal on the side contralateral to the lesions. These animals also show a tactile deficiency involving the exteroceptors in the hair follicles which is limited to the side of the animal ipsilateral to the lesion. A similar deficiency on the contralateral side is noted after unilateral lesions in the tectum. These experiments indicate that an afferent pathway for the sexual responses runs through the cord within the posterior quadrant, decussates at some point below the inferior colliculi, and runs through the roof of the mesencephalon.

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RESPONSES FOLLOWING ELECTRICAL STIMULATION OF THE CEREBELLAR CORTEX IN THE NORMAL CAT*

SAM L. CLARK

Department of Anatomy, Vanderbilt University School of Medicine, Nashville, Tennessee

INTRODUCTION

IN 1903 Lewandowsky described, incidental to some experiments of a different type, a method developed by Ewald (1898) for stimulating the cerebellum in conscious animals. In the skulls of 5 dogs he placed an ivory button containing two exteriorized wires which made contact with the cerebellum. The dogs were allowed to live a few days and were stimulated while unrestrained and unanaesthetized. Though his results from stimulation were inadequate, Lewandowsky observed a response during stimulation similar to that we have observed from the paramedian lobe, and from his description it is probable that this was the area stimulated in each of his 5 experiments. It is regrettable that this method lay so long unused, for it was apparently unnoticed by subsequent investigators, and it was not until the present experiments were completed that either the work of Ewald or Lewandowsky came to our attention.

Much has been learned by physiological and comparative anatomical studies of the connections and functions of the cerebellum (Larsell, 1937; Fulton and Dow, 1937). From the standpoint of the present report the most significant early work on stimulation was done by Ferrier whose book (1886) contains an excellent account of the work on the cerebellum up to that time. More recently cerebellar stimulation has been carried out largely on decerebrated animals and has been shown by several investigators to cause an inhibition of the antigravity muscles (Denny-Brown, Eccles, Liddell, 1929). Occasionally experimenters have stimulated the cerebellum in intact animals, but only a few after eliminating anaesthesia. Magoun, Hare and Ranson (1935) faradized the cerebellum of the intact monkey with the stereotaxic instrument, but under anaesthesia. The same workers later (1935, 1937) stimulated both normal and decerebrate cats and monkeys either under anaesthesia, or after recent anaesthetization. Mussen (1927 to 1934) reports experiments in which the cerebellum was stimulated in anaesthetized and a few unanaesthetized animals, employing with the latter a method similar to the one we have used. Brogden and Gantt (1937) also stimulated the cerebellum in normal, unanaesthetized dogs with an implanted electrode fed by a subcutaneous coil, after the method described by Loucks (1933) and developed further by Chaffee and Light (1934). They were not primarily inter-

ested in exploring the cerebellum but in the study of "conditioning" to cerebellar stimulation.

None of the workers mentioned has observed completely the phenomena to be reported in this paper, but each has made observations which fit into the general scheme. From our experience in stimulating the cerebral cortex (Ward and Clark, 1936), it appears probable that the discrepancies between the various reports are mainly dependent upon two factors, *viz:* the influence of an anaesthetic, and the control of the stimulating current. While some investigators have eliminated the anaesthetic, the physical character of the currents used for stimulation has had little attention.

METHOD

Electrodes were fastened permanently into the skull of a series of cats (Nembutal anasthesia); in each case the electrode was fixed over some previously determined point on the brain. The electrode consisted of a stainless steel tube with a tapering threaded end, containing in its axis an insulated silver wire. The wire was fused into the middle of a glass rod and this encased in a rubber tube, all of which fitted tightly in the steel tube. The slightly exposed tip of the silver wire embedded in the glass rod was ground smooth, so that it could not injure the brain. The tube was screwed into a trephine hole far enough into the skull to reach the surface of the cerebellum. The end of the silver wire which was in contact with the brain through a hole in the dura mater, was the stigmatic electrode, the steel tube serving as the indifferent electrode. By means of a detachable extension cord, controlled stimuli were applied to the brain through the outer end of the electrode, which projected through a hole in the skin. The voltage of the stimulating current was controlled through a voltmeter and the length of the stimulus was determined by a timing device. In these experiments a 60-cycle sine wave current, obtained from the lighting circuit by passing it through a transformer and a variable rheostat, was used consistently as the stimulating current. The cats were unrestrained throughout the experiment. Since the effect produced from a stimulus of 4 sec. may last as long as 10 or 15 min. it is obvious that stimuli must be properly spaced if the true effect of isolated stimuli were to be observed. Not all stimuli produce visible effects of such duration, but if a long effect appeared it usually began within 90 sec. after the stimulus. In these experiments, therefore, it was the custom to allow 2 minutes or more between successive stimuli.

Three people conducted the experiments as a rule. One adjusted the apparatus and checked the time at which different events occurred, one watched the cat and dictated a detailed description of each effect as it occurred, and a third took down in shorthand the description and recorded the strength and duration of the stimulus and the times at which the changes in the cat's movements occurred. Moving pictures were made of the cats during some of the experiments for recording and for checking the dictated descriptions. In this series 78 cats were used on which a total of 94 electrodes were planted (Fig. 1).

Unless something occurred to loosen the plug in the bone, the animals survived for days or weeks and could be stimulated at will. There was no appreciable reaction about the point which touched the brain; in fact, it was usually necessary when the animal was killed to unscrew the electrode and insert a bristle at this location into the brain before removing the bone so that the point of stimulation could be found. When removed, the brains were preserved in formalin and the location of the electrode recorded. References made to the "mid-line" of the vermis refer to an S-shaped line following the highest point of the ridge which the vermis forms, and not to a mid-sagittal plane. The extent of flexures of the vermis varied from animal to animal.

RESULTS

Stimulation of the cerebellar cortex through implanted electrodes in the unanaesthetized, unrestrained cat, results in movements which vary with the position of the electrode on the cerebellum, with the strength and duration of the stimulating current, and with the immediately previous experience of the

animal. There are, no doubt, other variables as, for example, the type of stimulating current, the residual effects of anaesthesia, the effect of pre-existing posture, etc. Under reasonably constant conditions the type of response from a particular cerebellar area is constant from day to day in the same cat, and the response is similar in different cats when corresponding areas are stimulated. The typical response obtained in these experiments from cerebellar stimulation can be divided into three phases for convenience of descrip-
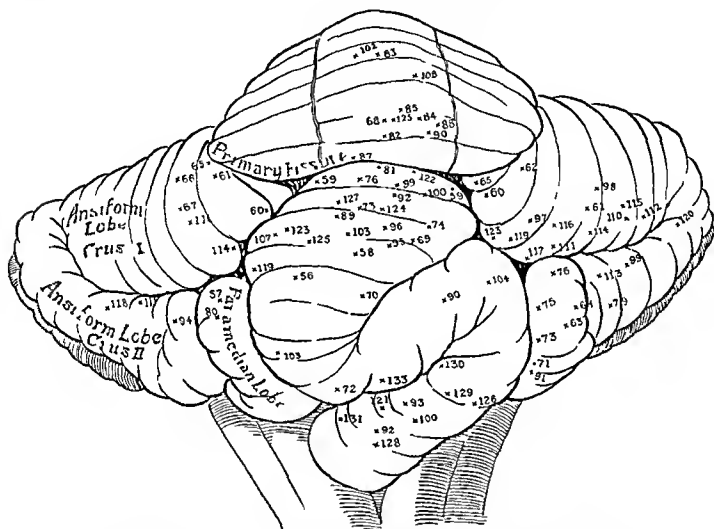
FIG 1 Diagram of a cat's cerebellum from the dorsal surface The serial numbers of the cats used appear adjacent to a small x which indicates the location of the point stimulated The S-shaped vermis varies in the extent of its flexures in different animals so that the location of some points on the diagram is only relative

tion There is first the phase of the response which is coincident with the stimulus and brief in duration. Immediately following is the second phase, ordinarily beginning on cessation of the stimulus, usually longer in duration than the first phase, more rapid in the onset and, in terms of movement, opposite in direction. Then the third phase, which may appear as a slow continuous transformation from the second phase, or may begin after a pause of as much as a minute or more. This phase progressively involves the head, limbs, body and tail of the animal in a series of slow movements lasting for several minutes. In this paper these three phases are referred to respectively as the phase of stimulus, the rebound, and the long after-effect.

The movements produced in these experiments differ in character from the clonic portions of epileptic attacks resulting from cerebral stimulation since they are in the main tonic and slow, resembling the movements in 'slow motion' pictures. The animal actually assumes a series of statuesque postures each of which builds up gradually to a maximum, and then fades as it is replaced by the succeeding pose according to a sequence which is definite for each area stimulated. The duration of the long after-effect, and the extent
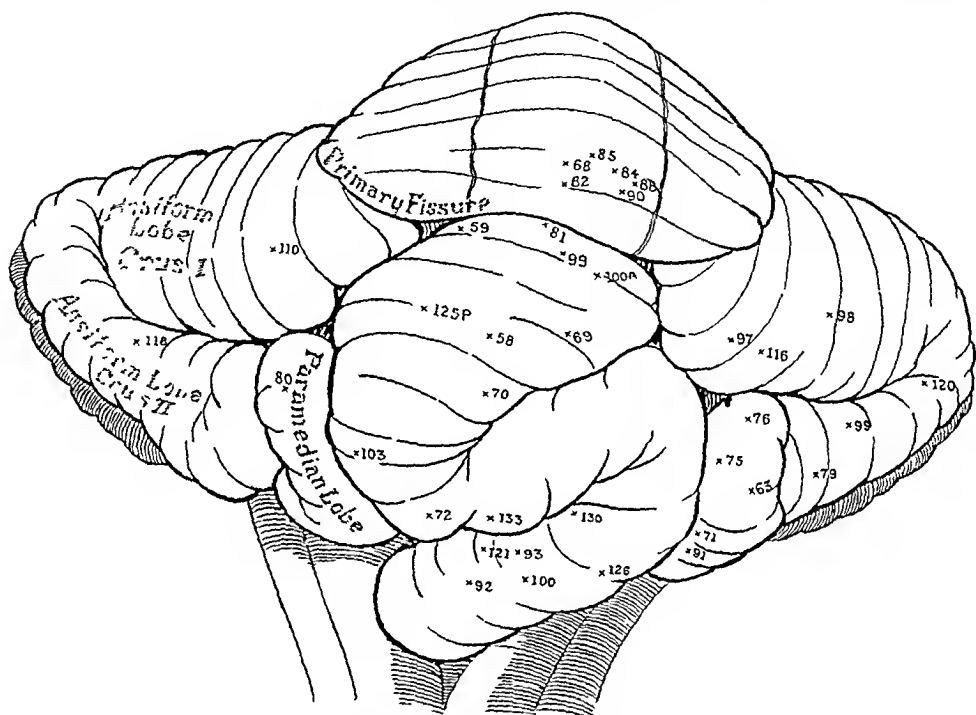
FIG. 2. Diagram of cerebellum showing the location of points on cats specifically referred to in the text as examples. The other points, omitted for the sake of simplicity conformed to the general scheme in their responses.

of involvement of the animal's parts, as well as the temporal relation of involvement of different parts, can be varied with the strength of the stimulus. A discussion of the variations in results from different strengths of stimulus can best be discussed after a typical experiment is described.

When a point on the margin of the vermis just back of the primary fissure (Fig. 2, 3, and 4) is stimulated with an adequate stimulus (2 to 5 volts for 4 sec.) the cat exhibits a series of movements lasting from 5 to 15 min. (average about 8 min.). With the stimulus the head turns gradually to the homolateral side, being held in this position for the duration of the stimulus. Promptly at the end of the stimulus the head turns toward the contralateral side in rebound. At the same time or within a few seconds afterward the homolateral forelimb may lift and protract; and may deviate in the direction of the head (Fig. 3). The foot may wave slowly and even be lifted higher than the head.

After about half a minute or a minute the animal returns to its normal resting posture or may go directly into the long after-effect. If the homolateral limb did not lift at first it now goes through the procedure. At this time the head may or may not turn tonically to the homolateral side. The forelimb gradually relaxes, while the contralateral forelimb becomes involved in a similar manner, and the head may turn to the contralateral side. By $2\frac{1}{2}$ to 3 min. after the stimulus these movements have subsided. There may appear a concavity of the trunk to one side then the other along with or following the forelimb involvement. About 4 to 5 min. after the stimulus the homolateral hindlimb
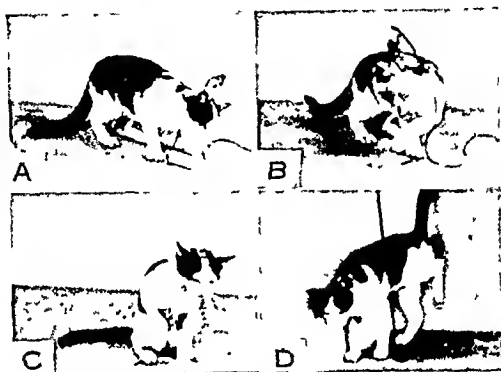
FIG 3 A to F (Fig 3 and 4) A series of frames from a moving picture taken of cat 125 following a stimulus of 4 volts for 4 sec to a point on the left side of the vermis 3 folia back of the primary fissure A, during the stimulus, the head slightly to left B, about 3 sec after the stimulus, the head of the cat is to the right in rebound and the left forefoot is being held up C, 3 75 min after the stimulus, the right forepaw being held up D, 7 min after the stimulus the left hindlimb is being lifted and retracted

begins to be affected, and lifts as did the foreleg showing an overaction of different groups of muscles in the extremity, so that its position gradually changes. It may be protracted awhile, then retracted, and the hocks may deviate medially then laterally. The tail at this time tends to curve tonically toward the homolateral side so that it is in a horizontal plane with tip pointing toward the head. These effects gradually give way and at about 6 or 7 min. after the stimulus the contralateral hindleg shows an effect like the homolateral (Fig. 4); the tail hooks to the contralateral side, sometimes showing a stage where it hooks dorsalward and at other times resembling a corkscrew in the period of transition from one side to the other. As these effects gradually cease the animal returns to normal.

In between the phases of such an attack there may be short intervals in which the animal stands or sits quietly or exhibits some voluntary or reflex *activity such* as eating or scratching or licking itself. There is, however, an obvious quieting of the animal until the effect of the stimulus is over, and the animal often appears as if preoccupied. In between the periods of marked limb involvement the cat may turn in small circles in the direction indicated by the head turning or concavity of the body. If the animal walks or jumps
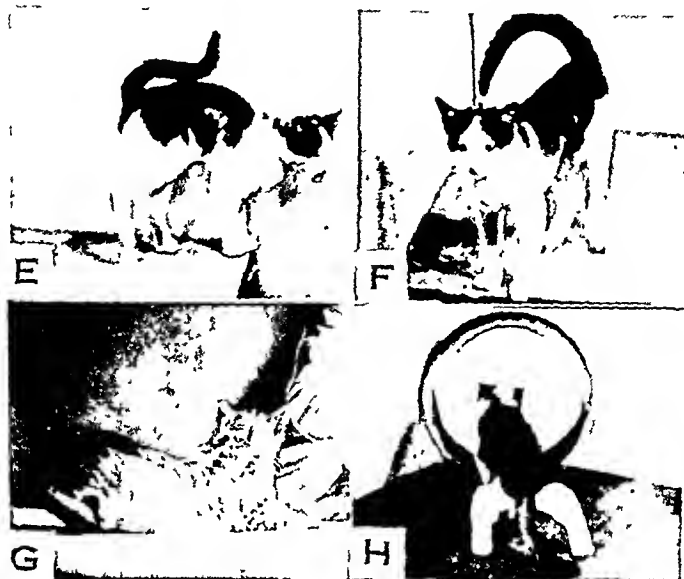
FIG. 4. E. 8.5 min. after the stimulus the right hindlimb is being held up and the tail is curved over the back. F. 8 min. and *50 sec. after the stimulus, the hocks are still turned in and the* tail is curved to the right. Later the tail turned left and the cat lay down on its right side. G. Cat 60, showing lifting of right hindleg with cat still lying prone, as part of the after effect of a stimulus to an electrode on the right side of the cerebellum. H. Cat 98, 3.5 min. after stimulation showing the way the hocks turn outward.

while the limbs are involved, an obvious dysmetria is manifest in the movements of the affected extremities.

Such is the typical history of a long effect when the point on the vermis is not in the midline. This is the type of experiment described in a preliminary report (Clark, 1937). It appeared likely from these results that a stimulus applied to a point in the midline might involve the animal in an attack in which the limbs of both sides would be simultaneously affected. This was found to be true, though due to the difficulty of planting a point exactly in the median plane, there is usually a slight lead of one or the other extremity, or a turning of the head to one side then the other. Following is a protocol from such an experiment:

On September 13 a plug was planted near the midline in cat 81, the point

proving on subsequent examination to be just on the posterior margin of the primary fissure, slightly to the left of the midline (Fig. 2). On September 15, after some preliminary stimulations of less intensity, a stimulus of 5 volts for 4 sec. produced the following: slight retraction of the head; when the stimulus ceased, there was a sudden rebound as the cat's head tucked down beneath its chest, the top of the head nearly touching the floor. This gradually subsided and the cat then slowly stood higher in its fore quarters. At 1 min. 20 sec. after the stimulus the left foreleg was lifted in high flexion. At 1. min. 35 sec. the right leg lifted once, the head turned to the right, and at 1 min. 45 sec. both forelegs were lifted from the floor and the cat sat on its haunches. This posture was maintained for about 20 sec. and first one then the other foreleg was placed on the floor. As one foot was put down the other was lifted. This continued until 2 min. 35 sec. after the stimulus. At 3 min. after, the cat walked cautiously forward with evidence of overstepping in the hindlegs, especially the left, which was lifted too high with each step. At 4 min. 20 sec. the cat lifted its right hindleg in high flexion, and walked, hopping on its left hindleg. This continued until 5 min. 40 sec. after the stimulus when the right leg was lowered. At 6 min. after the stimulus the cat stood with feet bunched in a small area. The hocks were turned in, the tail turned to the left, and the hind quarters crouched low. At 6 min. 30 sec. the tail turned to the right and the hocks touched in walking. At 7 min. 30 sec. after the end of the stimulus the tail turned to the left, and then the cat quickly returned to normal.

The patterns of response in such a long effect vary with the region of the cerebellum. The head movements are especially interesting when the point is near the midline. When the point is just rostral to the primary fissure (cats 68, 82, 84, 85, 88, 90, Fig. 2) during the stimulus the head goes down toward the floor (and sometimes forward), and in the rebound rises high (and sometimes backward) in the opposite direction, with perhaps deviation to one side and then the other as well. Back of the primary fissure (cats 58, 59, 81, 99, 100A, 103, 72) the effects of stimulation are just the reverse: during the phase of stimulus the head rises high, and in rebound the head is carried down. With the movements up and down may be combined horizontal movements of the head so that the head moves up and backward with the stimulus, down and forward in rebound, and perhaps deviates first to one side and then the other.

When the point is on the paramedian lobe (cats 63, 71, 75, 76, 80) on stimulation the animal leans to the opposite side and simultaneously lifts the homolateral forepaw. At the end of the stimulus there is no marked rebound, the animal merely returning to its original position; thus far we have been unable to obtain evidence of a prolonged after-effect with lifting of all extremities from any of the points planted on this area. The homolateral forelimb may show some effect, e.g., overstepping and disturbance of the placing reactions for a minute or more. There is a tendency for the cat to cringe when the paramedian lobe is stimulated. This is the only region giving much evidence of disagreeable reaction; the cats frequently purr throughout an experiment.

The results of stimulation of points near the vermis on Crus I of the ansiform are similar to the effects obtained from the vermis. Further away from the vermis, as in cats 97, 98 and 120, though the effects upon the forelimbs were separated in point of time of involvement during the long after-effect, the hindlimbs tended to become involved simultaneously. However, in cat 116, in which a plug was planted on the right ansiform (Crus I) on June 27, a stimulus on June 30 gave the following sequence in the long after-effect; the right foreleg was involved for 2 min., then 20 sec. later the left foreleg became involved, and was affected for about 1 min.; then at 4 min. after the stimulus the *left* hindleg began lifting and showed evidence of involvement for more than 2 min., overlapping the effect on the right hindleg which began to be involved at 5 min. after the stimulus. There was, therefore, a reversal of order in the effect on the hindlimbs as compared with the sequence obtained from points on the vermis, and this was observed on three different occasions. A similar reversal appeared once in cat 97, whose point of stimulation was in the same region as that of 116, and once in cat 70, whose point was on the right margin of the vermis considerably medial to that of the other two cats. This response from cat 70 was of further interest since the long after-effect was delayed in beginning, the right foreleg not lifting until 3 min. 30 sec. after the stimulus.

The effects from Crus II differed somewhat from those obtained from Crus I of the ansiform lobe. Points on the medial portion of Crus II produced effects similar to those on the nearby paramedian. Further laterally, as in cats 118 and 99, the long after-effect appeared, with the forelimbs being affected in the usual sequence (homolateral, then contralateral) but with the hindlimbs being involved practically at the same time, as in Crus I. It was in the ansiform lobe that effects on the eye and ear were observed. In cat 118, for example, with the stimulus the homolateral eye closed and the homolateral ear flattened, the opposite ones were similarly affected about 4 min. after the stimulus.

The effects obtained from points on the most posterior exposed portion of the vermis (92, 93, 100, 121, 126, 133) gave evidence of another variation in the general scheme. With gentle stimulation, if the point was not in the midline, the cat leaned toward the side stimulated uncovering the opposite hindlimb if seated, and it might abduct and extend the opposite hindlimb as if to prop the body. As the stimulus ceased the rebound occurred and is the mirrored image of the first effect. That is, the cat leaned toward the opposite side, withdrew the extended hindleg and placed the homolateral one in the propping position. With a weak stimulus, the seated cat might, as it leaned with the stimulus, make one hopping adjustment of the homolateral foreleg to support it in the leaning position. The leg returned to a natural position at the end of the stimulus.

These movements have the appearance of normal adjustments of the body to changes in the tension of groups of muscles, and, indeed, their counterpart can be produced by placing the hand on one side of a standing cat in the region of the thigh (or even of the shoulders) and pushing gently towards the opposite side. The cat leans against the pushing hand and abducts the

contralateral hindlimb as a prop to keep from being pushed over. If a quick change is made and the cat pushed from the other direction, it changes its position as it does in rebound to a stimulus. If a weak stimulus in this region actually produces a response from the cat comparable to that obtained by pushing the cat's body gently towards the contralateral side, then the effects of a stronger stimulus would not be unexpected, for a stronger stimulus caused its hind quarters to tend to fall (or actually fall) toward the contralateral side, and rebound at the end of the stimulus toward the homolateral side. In the case of the stronger stimulus the cat appears unable to adjust to the new situation until it has fallen in the direction indicated—just as with a stronger push it falls before it can adjust itself.

When the stimulus in this posterior part was in the midline, the cat did not fall in either direction but sank to the floor and extended its claws as if holding on. At the end of the stimulus it might rise again or lean towards one side. As yet we have not obtained much evidence of a long after-effect from the more posterior region of the vermis, though from cat 100 an effect of 1 min. 45 sec. involving tail and body occurred, but there was not the high lifting of limbs. From a point slightly anterior to this on cat 130 a long after-effect of about 8 min. duration occurred, involving all limbs and tail in the usual sequence.

Certain phenomena common to the long after-effects are obvious as one watches those obtained from various places on the cerebellum. First, there is the slow character of the movements elicited, common to all parts involved. The change from stimulus phase to that of rebound is usually rapid, but there follows a posture which is tonic and subsequent changes are slow. Furthermore, while the pattern in which portions are involved differs somewhat with the point stimulated and while the stimulus and rebound phases may differ in direction and character, movements of specific parts obtained from stimulation of various areas of the cerebellum have certain common qualities.

In the case of limb movements, for example, there is usually evidence that an extremity is becoming involved by the extension of the claws or extension and retraction of claws as in the kneading movements of cats (the 'pleasure reaction'). Then there is a tenseness in the extremity and a slow lifting. The lifting is interrupted for awhile by alternate periods of relaxation and the foot returns to the floor only to lift again until it is held at its highest point. There is a corresponding adjustment of the other parts to balance the animal. The position of the forelimb in its extreme state of lifting varies with the animal (apparently dependent on the point stimulated and the strength of the stimulus). It may be over the head, or extended in front of the animal or held flexed close to the chest wall. Often the lifted foot waves slowly for awhile. As the foot is returned to the floor it reverses the effect seen in the beginning. That is, the foot is dropped slowly and then lifted again, dropping more with each movement, but when it reaches the floor it may be lifted quickly again and again as if the floor were too hot to rest upon. There is at times evidence of hypersensitivity to touch in the involved extremity, and it will withdraw

suddenly on being stroked. When two extremities are involved nearly simultaneously each shows the same effect, and the one first involved may lift, only to be suddenly replaced on the floor for the opposite one to lift. As mentioned above it is possible for the cat to lift both forefeet at once (*e.g.*, 58, 68, 69, 120) and sit on its haunches for half a minute more or less. When this occurs, the extremity which first showed the effect also loses it first; and the cat will tentatively replace that foot on the floor leaving the other one lifted until the effect dies out in it.

Not only is lifting a characteristic of the limb movement, there are abduction and adduction, protraction and retraction, internal and external rotation, especially noticeable in the hindlimbs as the cat walks during a response. Frequently the hindlimbs were raised too high with each step and kickback before the foot descended, so that it landed almost in the place from which it was lifted and little progress was made. When both hindlimbs were involved the gait of the animal was strikingly like that which we observed (Ward and Clark, 1938) in a few animals following the clonic phase of epileptic seizures elicited by electrical stimulation of the cerebral cortex. Both before and after the stage of marked lifting of the limbs, the limbs involved showed dysmetria as the cat walked or jumped. One cat, jumping from the floor to a table toward the end of a long after-effect, twice jumped about six inches too high; this being the only visible evidence that the hindlimbs were still involved. Shortly after this the cat jumped to the table with its usual smoothness and accuracy.

Throughout the long after-effects it was obvious that the movements that occurred were paired in opposite phases; just as is the case with the movements of the stimulus and rebound (Fig. 5). For example, the head would turn to one side for awhile and then turn to the other; the head might be held back and high up, but shortly afterward it would go down and forward (or vice versa); the body would be concave to the one side, then to the other; the shoulders would be high, then low, the tail would be turned first to one side and then to the other, or it might hook ventralward between the cat's legs and then curved dorsalward over its back (at times of transition in attempting to turn to both sides at once the tail may resemble a corkscrew); the cat might stand high on its hind quarters and then sink low; the hocks may be turned in so that they touched as the cat walked and later be turned markedly out (Fig. 4). In some animals the ear was flattened on the homolateral side and the homolateral eye closed, while the contralateral ear and eye were not affected until later (cats 110, 118). All such movements occurred slowly, consuming from a few seconds to more than a minute just as in the case of the lifting of the limbs. And in each cat these movements occurred in a definite sequence so that having witnessed a long effect from stimulation of a certain point, one can predict with reasonable accuracy just when each movement will occur after a succeeding stimulus. Not only is the sequence the same but the time after the stimulus at which one may expect a phase of movement is approximately the same for stimulations of comparable intensity.

There was an optimum stimulus for the production of the long after-effect, and it was usually of greater strength than that necessary to produce only the phases of stimulus and rebound. As stimuli at a point were gradually increased, a threshold strength was reached at which there was only a slight effect during the stimulus and a slight rebound or, since the rebound was usually more conspicuous than the stimulus phase, there may be no visible effect during the stimulus, but the rebound nevertheless follows. Then, as
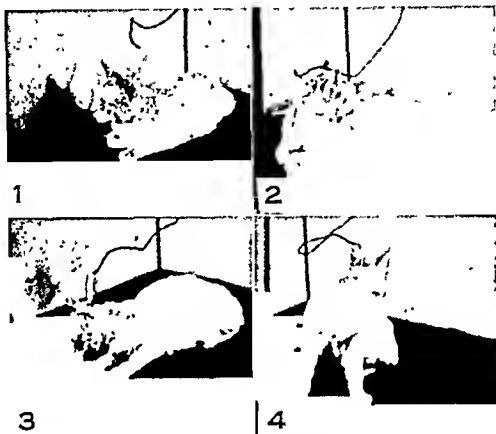
FIG. 5. Cat 95, whose point was on the right side of the vermis 3 folia back of the primary fissure. Pictures 1 and 2 show respectively the phases of stimulus and rebound following a weak stimulus. Pictures 3 and 4 show similar phases following a stronger stimulus, with obviously more marked effects. In 1 and 2 the head is turned slightly to the right, in 3 and 4 it is turned markedly to the left. The wire lead used in stimulating is shown attached to the electrode.

the stimulus was increased further, the motion in the first two phases became greater (Fig. 5). With still greater increase there might occur the phase of stimulus, followed by the phase of rebound, followed by only the first part (more or less) of the long after-effect. When the optimum strength was reached there occurred the three complete phases just described. If this optimum strength of stimulus was surpassed, the different phases might become telescoped, so that increasing the stimulus too far beyond the optimum tended actually to shorten the total duration of the visible effects.

Excessively strong stimulation not only caused a telescoping of the various effects obtained from a point, but it sometimes produced others as a by-product. For example, in cat 116 (right ansiform, Crus I) it caused the cat to fall to the right and the cat's tail rotated counter clockwise to one facing

the cat. A cat's tail will rotate in this direction if it is made to lean or fall on its right side. Too strong stimulation caused cat 85 (anterior vermis) to turn somersaults, and cat 79 (right ansiform, Crus II) to roll over two or three times towards its right. Occasionally (cat 59) after an optimum stimulus, when all parts of the animal have been involved in sequence, a new series of movements began with turning of the head and lifting of the homolateral forepaw, but as yet a second series has not continued beyond this. There has occurred, too, a repetition of the effect on the homolateral forelimb just after the effect on the contralateral forelimb (cats 60, 99 (middle), 100, 125P).

It is possible to mask or prevent the appearance of the long after-effect by stimulating at a strength that is too great, or by stimulating at intervals that are too close together, or by stimulating too recently after an anaesthetic. A long effect has not yet been obtained from an animal incompletely recovered from the anaesthetic; frequently more than 24 hours in the case of nembutal. After one or more successful stimuli it may not be possible to obtain the long after-effect until several hours have elapsed. However with cat 81, a day following implantation, three long reactions were evoked, each more than 7 min., within a half hour. And after several days of successful stimulation a point may cease to give the long after-effect though the stimulus and rebound phases persist. A rise of threshold usually accompanies such a change. Small injuries to the cerebellum in the neighborhood of the point sometimes appeared to abolish the long after-effect. In some animals, in which a technical fault occurred, a long after-effect may not be obtained from a region known to produce such effects, though the first two phases occur.

Deviation of the eyes toward the side stimulated has occurred in some cats during the stimulus, and was associated with or preceded the turning of the head in that direction. Likewise, in the phase of rebound, the eyes may deviate to the opposite side accompanying or preceding the turning of the head in that direction. Nystagmus was not uncommon following the stimulation of the cerebellum, and was observed once, with weak stimuli; it was replaced by constant deviation following stronger stimulation. The observations of eye movements and changes in the pupils have been limited by the necessity of watching other movements. We have not observed contraction of the pupils as did Ferrier, and Hare, Magoun and Ranson (1937) on stimulation of the cerebellum, though dilatation has been frequently seen. No other effects on autonomically innervated structures have been noted except that in some animals a fluffing of the tail accompanied an after-effect following a strong stimulus.

Circling has appeared in most of the long after-effects, usually just following the rebound phase, but sometimes later. The cat, having turned its head toward one side, merely walks around in that direction one or more times in a small circle. Some animals during the circling may lie down on the convex side just as a cat does normally when it curls up to rest. Other examples of such 'opportunism' appear occasionally. A cat may utilize the position of its turned head to lick the nearby shoulder, or the upraised paw may be used to

rub the back of the head (the head being free from involvement at the time and taking the initiative).

The responses evoked, while influenced to some extent by the position of the cat, may cause spontaneous activity to cease. Cat 60, for example, was stimulated while playing with a string. The play was interrupted by the artificially induced movements of the forepaws. A cat that is eating will stop while the influence is affecting the head and fore quarters but may eat normally while the hindlimbs are affected. A cat lying in a sphinx-like position will still lift a hindlimb without rising when that limb becomes involved (Fig. 4). On the other hand, when the effect is weak in an extremity spontaneous activity will mask it, or obliterate it during a voluntary movement.

DISCUSSION

While the results described in these experiments from cerebellar stimulation are not identical with those of any previous investigator, many of the stages of special movements which are reported here resemble markedly portions of experiments described by others. For example, our observations of deviation of head and eyes agree in general with those of Ferrier and of Bechterew (as cited by Tilney and Riley). Horsley (1906) has reported that he and Clarke were unable to elicit movements from stimulating the cerebellar cortex but the movements from stimulation of the central nuclei are not unlike some we have obtained from the cortex. They observed deviation of the head and eyes to the same side from one area, and powerful bicipital flexion of the elbow from another, etc. They report the eye movements as steady with no clonic intermission and no after-effect when the stimulus ceased. The familiar effect of stimulating the cut surface of the mesencephalon as described by Graham Brown (1913, 1915) has much in common with certain stages in the movement from stimulation of the cerebellar cortex: homolateral bending of head and tail, flexion of the homolateral forelimb, etc. It is significant that Graham Brown observed that the posture was often maintained after the stimulus ceased; the ipsilateral flexion (or contralateral extension) outliving the evoking stimulus several minutes. And he found that stimulating the contralateral red nucleus during the phase of after-discharge produced a rebound phenomenon.

Denny-Brown, Eccles, and Liddell (1929) in addition to inhibition of the antigravity muscles in decerebrate rigidity, observed that in animals in which the level of decerebration is high, cerebellar stimulation may elicit "sharp flexion of the elbow joint, flexion (dorsi-flexion) of the wrist, abduction and extension of the digits and protrusion of the claws." They describe the position of the forelimb as resembling that of the 'rampant' animals of heraldry. Further, they observed that "after and sometimes during the stimulation the limb 'strikes' by the elbow becoming extended, the wrist and fingers flexed." Mussen has described specific responses from small areas of the cerebellar cortex. While we have not confirmed the details of these experiments, many

of the movements he describes we have observed in the course of long after-effects.

The descriptions of the effects of cerebellar stimulation with the stereo-tactic instrument on intact and decerebrate monkeys and cats given by Hare, Magoun, and Ranson (1937) contain points of extreme interest in application to the present experiments. They have observed movements in two phases (an inhibition of a posture or the assumption of a new position) during the stimulus, and a second phase following the stimulus, usually contrary to the first, appearing as a rebound. While they did not obtain the successive movements we have seen in the long after-effects, they observed the long duration (in some instances 5 minutes) of the effect of a stimulus. In addition to movements involving the trunk and limbs of an animal all at once, the effects on the homolateral forelimb alone are not unlike some of the movements we have obtained. There are a number of other features of their experiments which have much in common with the present report. The fact that the phase of stimulus and rebound which we have observed occur from stimulation of the cerebellar cortex is not so surprising, but in comparing the present results with certain previous reports of cerebellar stimulation, the direction of movement during the phases of stimulus and rebound from various areas is significant.

Both in the monkey and in the cat Ferrier observed that stimulation of the cerebellar vermis produced movements of the eyes. Stimulation anterior to the primary fissure caused the eyes to move upward, while stimulation back of the primary fissure caused them to move downward (with a deviation toward the homolateral side if the electrode was not in the midline). Ferrier states in connection with experiments on the monkey (1886, p. 189) "When the head is allowed free play the movements of the head coincide with movements of the eyes," and at times he observed involvement of the limbs. He pointed out that stimulation of this anterior portion of the cerebellum excites muscular combinations which would counteract a tendency to fall forward; and that stimulation of the vermis more posteriorly excites muscular combinations which would tend to prevent the animal from falling backward. "We should therefore expect to find," he continues (p. 199), "that a lesion which annihilates the functional activity of any of the individual cerebellar centers should manifest itself in a tendency to the overthrow of the balance in the direction naturally opposed by this center. This also is in accordance with the facts of experiment." That is, he found that destruction of the "anterior part of the median lobe (monticulus)" results in a tendency for the animal to fall forward; while destruction of the "posterior part of the median lobe (declive monticuli)" produces a tendency for the animal to fall backward (movements opposite in direction to those he obtained from stimulating these areas). Mussen (1931) later expressed similar ideas and reported similar results. Ferrier states that Flourens and Renzi in 1864 observed the effects of lesions on the vermis as described.

There is an apparent conflict between these statements and the observa-

tions reported here. Both Ferrier and Mussen stated that the effect of stimulating the anterior or posterior vermis was opposite, so far as the contracting muscles were concerned, to the effect of removal of the same part of the vermis. We have found, on the other hand, that the movement during the phase of stimulus of anterior or posterior vermis is similar in direction to the posture obtained on removal of the part stimulated while that in the phase of rebound is the opposite. An obvious corollary to this statement is that removal of one portion of the vermis (anterior or posterior) causes an effect similar to the rebound which follows stimulation of the opposite portion of the vermis in the intact animal. The accompanying table will illustrate this point.

Table 1. The anterior and posterior vermis compared

	Anterior vermis	Posterior vermis
Removal Flourens & Renzi Ferrier Mussen	Head held down (animal tended to fall forward, anterior neck muscles contracted)	Head held up (animal tended to fall backward, posterior neck muscles contracted)
Stimulus Ferrier Mussen	Head goes up (posterior neck muscles contracted)	Head goes down (anterior neck muscles contracted)
Present experiments	Stimulus—head down Rebound—head up	Stimulus—head up Rebound—head down

Evidently Ferrier and Mussen saw in stimulating the vermis only the phase of rebound seen in our experiments. Ferrier's statement is significant at this point (1886 p. 190), "It is also to be noted, in reference to electrical irritation of the cerebellum, that occasionally stimulation is absolutely without effect at first, and that after the lapse of some time the phenomena follow with great precision." It is possible that working with animals recently anaesthetized a stronger stimulus was necessary, and we have frequently observed that a stimulus stronger than optimum will cause the rebound phase to begin before the stimulus actually ceases; furthermore a brief but intense stimulus might give insufficient time for the appearance of the excitatory phase, but, on cessation, allow the appearance of the rebound. In the present experiments a stimulus about 4 sec. in length brought out the phases to best advantage.

The mixed representation of afferent fibers from different bodily areas in the same portion of cerebellar cortex is significant (this point is discussed by Hare, Magoun and Ranson, 1937). Since the cerebellar cortex is nearer the afferent side of the reflex arc than the efferent side, if one measures distance by the number of synapses in the neuronal chain, stimulation must have something in common with the reception by the cerebellum of a mass of afferent impulses. A presentable theory could be built up which would explain the movements elicited after electrical stimulation of the cerebellar cortex as

actual responses to an artificially induced sum of 'proprioceptive information,' the movements being identical with those that would follow from a posture that would present to the cerebellum a similar body of normal proprioceptive impulses. Much of the cerebellum remains to be explored, but we have stimulated directly some point on all but one (the vestibular flocculonodular lobe) of the four principle subdivisions into which Larsell (1937) divided the cerebellum from a functional viewpoint.

SUMMARY

Electrical stimulation of the cerebellum in normal unrestrained cats is followed by visible movements involving the various parts of the animals' musculature. Such movements may appear in three phases; the first with the stimulus; the second appearing as a rebound opposite to the first and immediately following the end of the stimulus; the third, prolonged and involving the various parts of the animal in a series of relatively slow movements in a definite sequence lasting several minutes.

The pattern of movements which may be elicited from the same point in a cat from day to day remains the same both with respect to the character of the separate movements and the time of their appearance in the sequence. Different large areas of the cerebellum respond to stimulation with patterns of movement having a recognizable specificity for the area. There is a certain common quality to the movements elicited from the cerebellum which is different from the movements that occur in clonic phases of epileptic attacks provoked by stimulating the cerebral cortex.

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SIMULTANEOUS ELECTROMYOGRAMS AND ELECTRO-ENCEPHALOGRAMS IN PARALYSIS AGITANS*

ROBERT S. SCHWAB AND STANLEY COBB

*From the Departments of Neurology and Psychiatry, Massachusetts General Hospital,
and the Departments of Neurology and Neuropathology,
Harvard Medical School, Boston*

IN AN EFFORT to discover the origin of the rhythmic tremor characteristic of post-encephalitic and arteriosclerotic paralysis agitans, simultaneous elec-
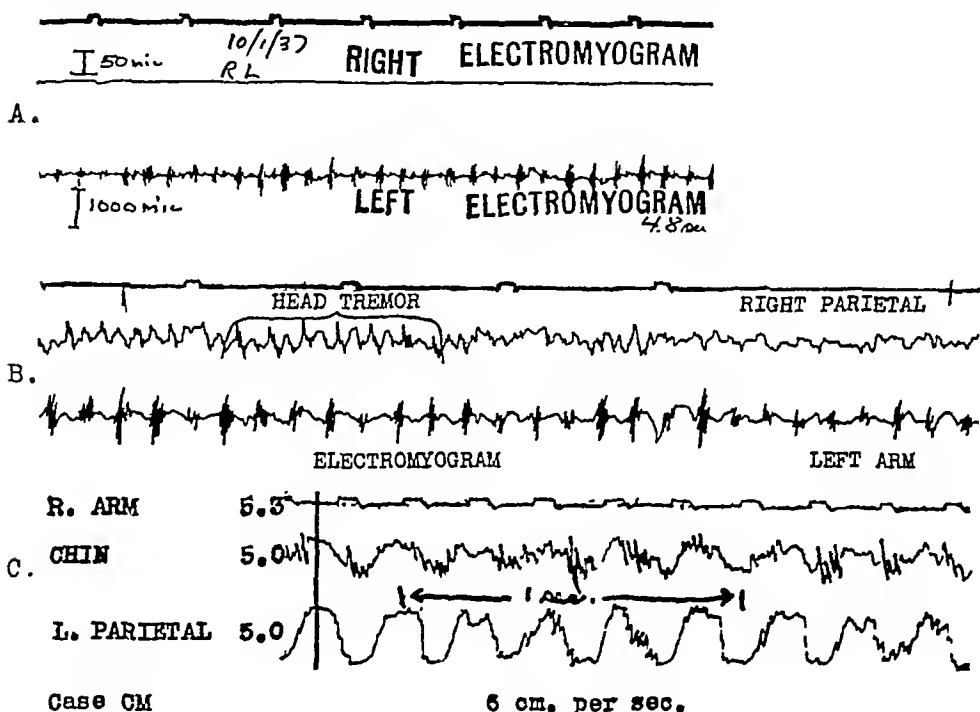
FIG. 1 A. Simultaneous electromyograms in right and left flexor digitorum sublimis in patient R. L., with left-sided paralysis agitans. Note there is no electrical activity in right arm which is a base line, free of artifacts with this degree of amplification from surface electrodes.

B. Simultaneous electromyogram and electroencephalogram in patient with severe bilateral paralysis agitans. The spikes that appear in the head lead as synchronous with the arm tremor are artifacts due to movement of the head electrodes.

C. Simultaneous electromyograms from chin, and arm (recorded through timing pen), and electroencephalogram, showing synchronous waves.

tromyograms and electroencephalograms were recorded from 37 patients. If the tremor originates from a rhythmic discharge in the brain, strong enough

in some cases to involve all extremities and dominate the motor activity of the individual, one might hope to be able to record it from electrodes placed on the intact skull. Discharges as slow as 4 to 6 per sec. might be expected to build up enough potential to be picked up, even if the seat of the disturbance is well beneath the outer cortical layers. The patients were selected from the Neurological Service of the Massachusetts General Hospital (Service of Dr. J. B. Ayer) and a few private patients of the authors. Fifteen of the 37 were classified as post-encephalitic and 22 arteriosclerotic, but the classification is not accurate as it was often impossible to get a history of encephalitis in the
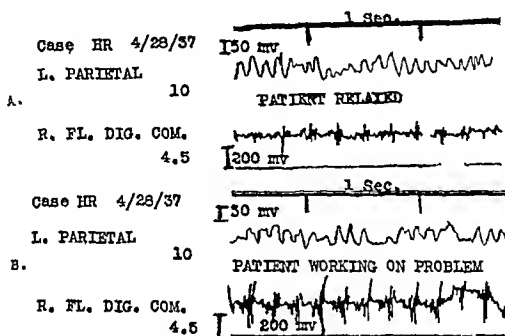
FIG. 2 Simultaneous electroencephalograms and electromyograms in patient H R A Patient relaxed B Patient working calculation No correlation of two rhythms Note increase in amplitude of muscle rhythm with mental effort

younger group or demonstrate arteriosclerosis in the older one. Cases that did not have tremor were not studied. About half of the patients were not taking hyoscine or stramonium when studied; nine of these were observed again when on hyoscine or stramonium. The rest were taking one of these drugs.

METHOD

The apparatus was a two-channel push-pull set of amplifiers as described by F. and E Gibbs, made by A Grass of the Harvard Medical School The power amplifiers fed into a two-pen ink-writing oscillograph capable of following frequencies up to 120 per sec with three-paper speeds (1 5 cm , 3 cm , and 6 cm per sec. respectively). The electrodes for both skull and muscle were small solder discs, 6 to 10 mm in diameter, fused onto No 32 enamelled copper wire These were fastened on with electrode paste and adhesive or collodion placed over the electrode to hold it securely in place The patients were usually recumbent, but a few were in an armchair with a head rest The room was not sound proof. The patient was examined both with his eyes closed in the dark, and also with eyes open in the light. The usual procedure was to ground the muscle channel to get rid of the cardiac potentials and record the electroencephalogram on push-pull Records were commonly obtained from six skull points, right and left occipital, motor, and frontal areas For the

comparison of electroencephalogram with electromyogram in this series, the contralateral motor area was used unless otherwise specified. Simultaneous tracings from different extremities and from different muscle groups in the same extremity were also made. Records of both monopolar and bipolar leads were made.

OBSERVATIONS

Before regarding the recorded potentials from the intact skull as due to currents from the nervous system, it is extremely important to exclude the artifacts that may occur. Eye movements, muscle potentials, electrocardiographic tracings are now familiar enough to be identified and discarded. We
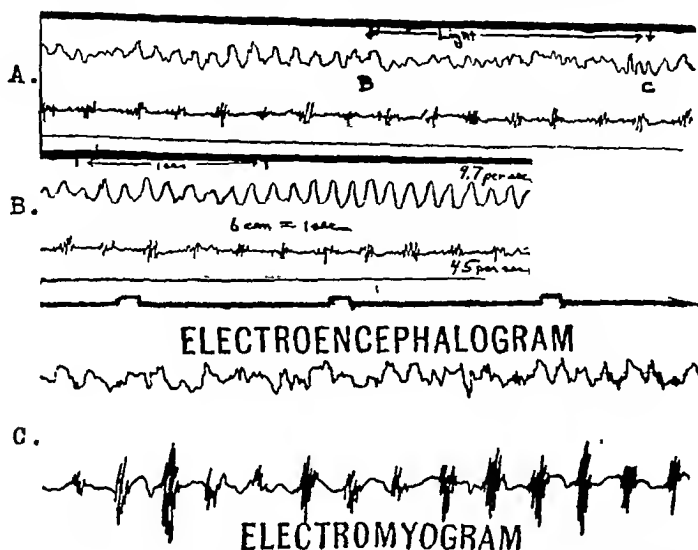
FIG. 3 A. Patient in dark, with eyes open, showing block of alpha waves by light from B to C, and showing no effect on tremor rhythm.

B. Same patient as A, in dark, with eyes closed, showing more marked alpha rhythm. No correlation in muscle and parietal cortical waves.

C. Another patient, showing no correlation between electromyogram and electroencephalogram.

noted in severe cases involving all extremities with obvious movement of the whole body at times, that spikes appeared in the tracing from the head leads which were synchronous with the tremor rhythm. Other observers have believed such spikes to be caused by cortical potentials related to paralysis agitans. We believed that they might be artifacts due to mechanical movements of the head electrodes set up by the tremor, (Fig. 1B). Electrodes on the chin or bridge of the nose in these severe cases produced this same sort of tracing indicating that the tremor spikes were artifacts. In order to confirm our suspicions in the same case as shown in Fig. 2, simultaneous chin and head leads were run into the two channels and the tremor of the arm recorded mechanically by means of a key and the timing pen. All three tracings showed synchronous spikes, proving them to be mechanical artifacts, whereas the

brain wave in this same case was of normal alpha rhythm when the head was quiet (Fig. 1C).

In none of our 37 cases were cortical waves synchronous or in any sort of phase relation to the Parkinsonian tremor of the rhythm. In 4 of the cases we found the head tremor artifact in the cortical leads at some time, and saw it disappear when the head was quiet. The cortical rhythms varied from 10 to 25 per sec., sometimes spontaneously or following bright light stimulus or emotion. The rate of the Parkinsonian tremor, however, remained relatively constant, and the maximum change per sec. being only 6 per cent, e.g., from
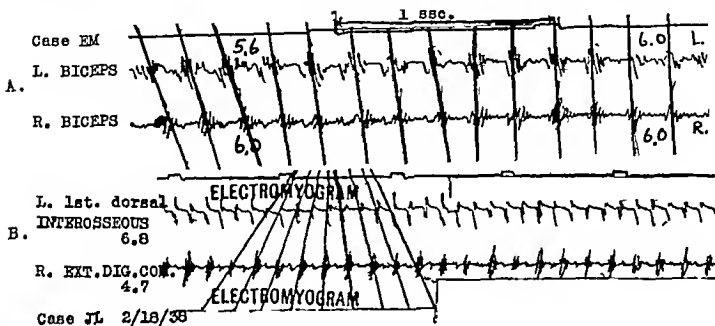
Fig 4 Simultaneous electromyograms on two different muscle groups A. Patient E M , showing a period of asynchronism followed by a period of synchronous discharge. B. Patient J L , showing complete lack of synchronism, even on repeated runs.

4.2 to 4.5 per sec. With rest and relaxation the cortical rhythm tended to decrease in rate and increase in amplitude, whereas the muscle tremor decreased in amplitude and did not significantly change in rate (Fig. 2A and B). If the average of the cortical rhythm in each case is plotted on a graph against the simultaneous average of the muscle rhythm, there is no correlation whatsoever (Fig. 5).

This study also showed that if careful counts of the tremor are made over 10-sec. periods, the tremor is not of exactly the same rate in two extremities, or even in two independent muscle groups in the same extremity (Fig. 4). Variations in one muscle group of 10 per cent (from 4.1 to 4.6 per sec.) could be found in an hour's observation. The cortical rhythm is more variable and shifts more often. Furthermore, the rate of the cortical potentials is not only different from the tremor rhythm, but bears thereto no simple numerical ratio. For example, if a patient relaxes, the amplitude of the muscle tremor diminishes and may even disappear for short periods, but there is no demonstrable change in cortical potentials, (Fig. 2A and B). On the other hand bright light or opening the eyes may change the cortical potentials without affecting the muscle tremor (Fig. 3A and B).

DISCUSSION

In 1922 Cobb[1] studied the rate and form of the tremor in paralysis agitans by means of a string galvanometer. He considered the rate to be remarkably steady for any one individual regardless of the size or location of the muscle under observation. Since the movements of the galvanometer string were recorded on short strips of film, extensive counts were not possible. In the present study, using an ink-writing oscillograph that permits observation of
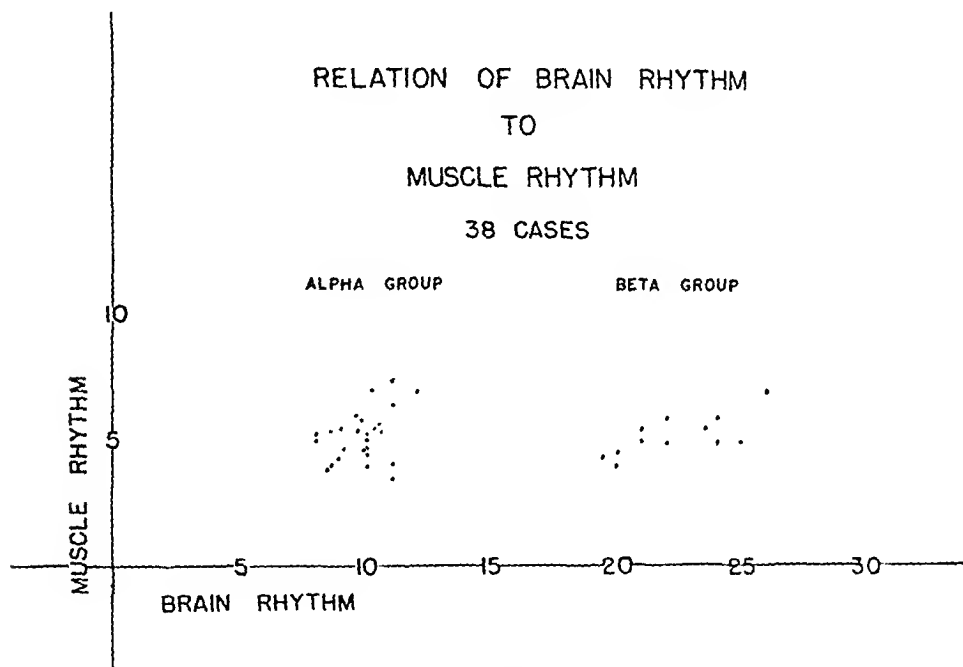
FIG. 5. Summary of the average rate of 10 sec. counts of the 38 cases. The brain rhythms are plotted along the horizontal line and form two groups of waves, the alpha (10-cycle) and beta (24-cycle). Muscle rhythms are plotted along vertical line. No correlation exists.

many yards of paper at a time, significant variations in the rate of the tremor of one muscle were found, and in two different muscles observed simultaneously. These variations are slight, but definite. Similar variations were observed by Herz[2] in 1931 by making cinematograph records of the trembling limbs of patients with paralysis agitans.

Recently, Jasper[3] has published data which he considers proof of the synchronism of the cerebral and muscular potentials in paralysis agitans. The observations described above and shown in Fig. 1B and C. we believe, explain these synchronous waves of Jasper as an artifact. This may also explain the slow waves observed by Yeager and Baldes.[4] If the potentials in the cerebral cortex and in the peripheral muscle were synchronous, one might postulate that the tremor had its origin in the cortex of the fore brain. Our data suggest that the tremor does not originate in the cortex, but in some nervous structure

at a lower level Since lesions in the motor nerves and in the motor tracts of the cord abolish the tremor of paralysis agitans, our electroencephalographic observations indicate the origin of the tremor lies in some nucleus of the brain stem or basal ganglia

CONCLUSION

In 37 cases of paralysis agitans simultaneous electroencephalograms and electromyograms failed to show any relationship between the two rhythms

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TEMPERATURE CHANGES IN THE CORTEX AND HYPOTHALAMUS DURING SLEEP*

H. M. SEROTA

Department of Physiology, University of Chicago

THE EXISTENCE of a "sleep center" is now established by animal experiments and clinical data supplemented by necropsy material. The localization of such a center has been much studied and the literature, adequately reviewed by Rowe, places it in the diencephalon, in the hypothalamus, or in the floor of the third ventricle. The mechanism of its function, however, has received little attention. Hess (1931, 1932) has stimulated the sleep area electrically through fine platinum electrodes embedded in the brains of otherwise normal animals and elicited true sleep, easily repeatable in the same animal. On the basis of this and other observations, such as pupillary constriction and increased intestinal mobility during normal sleep, he postulates the existence of a parasympathetic center which, by increasing its activity, leads to sleep. This implies the discharge from it of sleep-producing impulses.

In the human (Fulton and Bailey, 1929) and the cat (Ranson, 1934), however, sleep and somnolence are the usual outcome of destructive lesions in the same area. Further, Ranson has elicited rage reactions rather than sleep by hypothalamic stimulation. The seeming conflict in producing sleep by stimulation or destruction of a localized "sleep center" demands further study. It is conceivable that an actively functioning sleep center should manifest an increase in metabolism while other regions, *e.g.*, the cortex, show a decrease during sleep. The technique devised for the localization of thermal changes in the cat brain (Serota and Gerard) was therefore adapted to the study of this problem.

METHOD

Twenty-six cats were operated on aseptically under intraperitoneal nembutal anaesthesia, and two or three thermal needles inserted into the brain with the aid of the Horsley-Clarke instrument. Through small skin incisions and narrow drill holes the needles were lowered to the proper depth and then secured by sterile wooden wedges, dental cement, or Duco cement, spread uniformly within a hollow brass screw. The animal was allowed 18 to 24 hours recovery before study. Post-operative pain or infection was minimal, and most animals were active and ate within a day or a day and a half. Only docile cats with like weight and skull characteristics were used.

Thermocouples were made of 38 gauge enameled copper and constantin wire and were further insulated at the bared junction with Duco cement or Bakelite varnish. The extra-calvarial portion was covered with waxed linen, rubber, or gutta percha to guard against fortuitous fluctuations in environmental temperature. These insulated wires were connected to binding posts on a cap or collar fixed on the animal. From these, low resistance cables of the same wire connected the constantin, through a constant temperature junction, and the copper, directly, to the galvanometer circuit. The resistance of the thermocouples was 25 ohms, that of the galvanometer, 30 ohms. The sensitivity used in these experiments was 10 mm. $\approx 0.1°C.$, the galvanometer being slightly overdamped. While the

animal behaved in a normal manner, pacing about the cage, eating or sleeping, temperature readings of each area were made every 3 minutes, the shift from one thermoneedle to another requiring only 15 sec. The hypothalamic lead was placed within the following boundaries (Ranson): 6 to 12 mm. anterior, 0 to −5 mm. vertical and 1 to 4 mm. lateral. The cortical leads were variously inserted in the suprasylvian, lateral, and splenial gyri, from 16 anterior to 2 mm. posterior to the interaural plane, and from 2 to 5 mm. below the dura. The position was checked by gross section.

The criteria of sleep were: the assumption of the "sleep position" (LDCU, *i.e.*, lying down curled up, Fig. 1), and its maintenance for at least 20 minutes; respirations fewer than 20 per min.; few full-bodied movements or changes of position; and refractoriness to noise (*e.g.*, no response to dropping a metal weight on the table). By all these tests an animal nearly always slept shortly after a satisfying meal. Other occasional "naps" or resting periods were easily excluded.

FIG. 1. A typical variant of the "sleep position" in which the animal lies down curled up, "LDCU." (Photographed by Dr. L. L. Robbins.)

RESULTS

The hypothalamus is consistently 0.1 to 0.5°C. warmer than the cortex. This difference fluctuates considerably during the waking state but remains positive at all times. While the animal paces about the cage, sits, cleans itself, or eats, the temperature curves of these areas rise and fall as much as 0.2°C. The variations plot an irregular saw-toothed curve, each tooth lasting from 6 to 12 min. and at times recurring in a definite rhythm or coincident with gross skeletal movements, (Fig. 2). Other more regular temperature changes accompany definite behavior. Thus, during the obvious excitement which follows the sight and smell of food, the temperatures of both hypothalamus and cortex rise, but the former increases more. When food is eaten, both temperatures drop, and the change in the hypothalamus is again greater. (The temperature drop is a direct cooling effect of the food on the blood, for eating warm food leads to a rise.) Defecation and urination also result in a temperature fall. When the animal lies or crouches, both temperatures fall.

In all of these instances both structures appear to change temperature simultaneously.

During the somnolence which frequently follows a meal, while the animal nods and crouches, the temperature curves become irregularly rhythmical, both the temperatures and their difference (hypothalamus minus cortex) rising and falling by 0.1°C. over intervals of 6 min. Prior to the assumption of the "sleep position" there is usually a rise in the hypothalamic temperature, and when the animal curls up on its side and sleeps both the temperatures and the difference between them fall and the jags on the curve are replaced by a reflectively flat plateau (Fig. 2). The hypothalamic temperature may drop
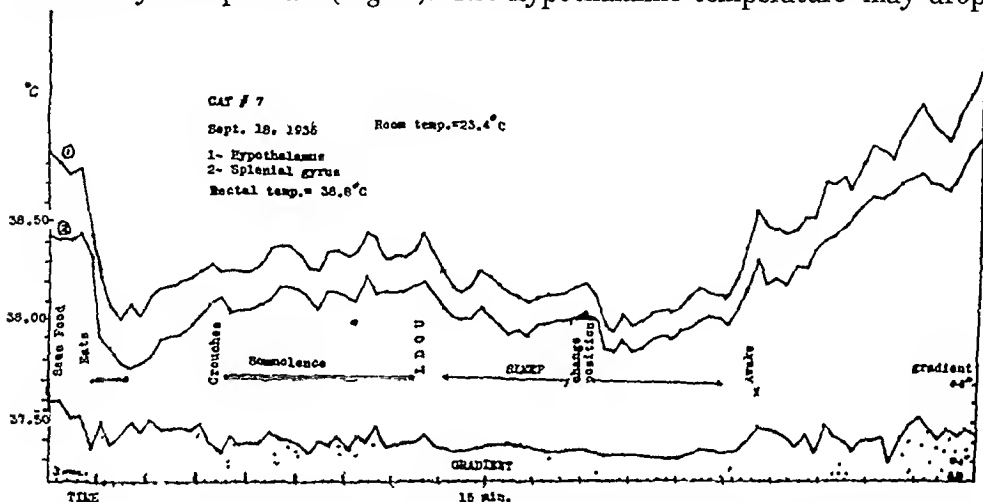
FIG. 2. Characteristic findings in sleep. Ordinate, temperature in degrees Centigrade; abscissa, time in 15 min. intervals. Two thermocouples, a cortical and hypothalamic, are simultaneously followed at 3 min. intervals. LDCU represents assumption of the "sleep position." Curve 1, absolute temperature of the hypothalamus; 2, same of the cortex. The stippled area is the temperature difference between them.

as much as 0.4°C. and the cortical only 0.2, so that the difference is reduced by 0.2°C. On waking the changes are reversed, and frequently a temperature increase, seen first in the hypothalamus, precedes the other signs of arousing.

The greater fall, during sleep, of the hypothalamic temperature than that of the cortex is not shared as markedly and consistently by other structures. Thus, a third needle in Ammon's horn or in the tail of the caudate nucleus showed a smaller temperature change than the hypothalamic needle. (These structures are at a depth equivalent to that of the hypothalamus and serve to control any effect of sleep on the pre-existing gradient (Serota and Gerard, 1938). Not all curves fitted the above description. Thus, of 119 curves on 26 animals, 88 showed a fall during sleep, 18, a rise, and 13, no change. About the same proportions hold for each lead. In 80 cases the curve flattened to the typical plateau, sometimes with a rise of temperature. On comparing hypothalamus and cortex the temperature in the former decreased relative to the latter in 31 cases out of 37 and rose in only 1. The temperature of the cornu

and caudate, in contrast, fell relative to that of the cortex in only 7 and rose in 3 cases out of 14.

To determine to what extent the specific cooling of the hypothalamus in sleep was due to diminished metabolic activity of its cells or to change in blood supply, the heated thermo-junction of Gibbs (1933) was used. This responds to greater blood flow by a drop in temperature larger than any changes in the unheated needle (Serota and Gerard, 1938). The heated needle
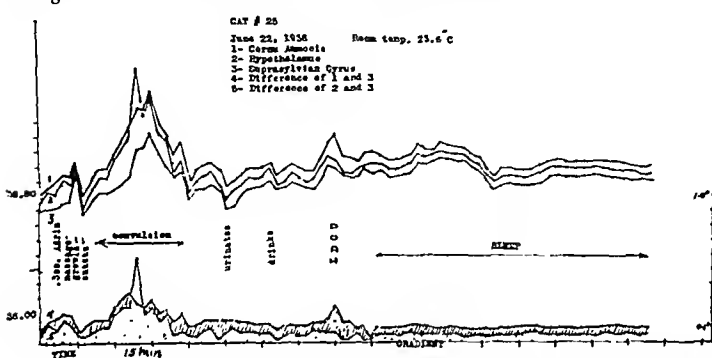
FIG. 3. Minor convulsion induced by epinephrin injection. Ordinate and abscissa as in Fig. 2. The temperature changes recorded by three thermocouples placed in the Cornu Ammonis, the hypothalamus, and cortex, are followed in curves 1, 2 and 3 respectively. Curve 4 represents the temperature difference of curves 1 and 3; and curve 5, of 2 and 3. Following the subcutaneous injection of epinephrin a period of hyperkinesis, ataxia and hypertonia results. This is succeeded by normal behavior and then by deep sleep.

showed only a slight temperature fall during sleep, indicating an insignificant change in blood flow. The validity of this conclusion was further checked by finding that eating either warm or cold food, which increases blood flow, cooled the heated needle, though the unheated needle was warmed or cooled, respectively, by warm or cold food, and by a smaller amount.

It follows that the hypothalamic neurones decrease their activity in sleep. It might be anticipated that in excitement the reverse would occur. Confronting a cat with a barking dog elicited in it the usual emotional storm and increased all brain temperatures, that of the hypothalamus most. A similar stimulation by epinephrin (0.3 cc. of 1–1000 Adrin subcutaneously, followed by local massage) caused hypertonia, ataxia and hyperkinesis amounting to a mild convulsion, and lack of response to a threatening gesture before the eyes. Subcortical temperatures rose more and sooner than that of the cortex and paralleled the motor symptoms, the hypothalamic temperature outstripping the others at the moment of most violent hyperkinesis. As the temperature difference between cortex and hypothalamus subsided, the convulsion ceased, the animal defecated, urinated, ate in the usual manner, lay down, and fell

into an unusually deep sleep (Fig. 3). (Compare the silent period of the EEG after an epileptic discharge.)

Hypothalamic temperature and not others, would frequently rise over a period of 3 to 12 min. during sleep and at its maximum the animal would suddenly shift position. Temperature then fell at once with the attainment of the new position. This continual accumulation of "tension" during sleep, released in muscular movement, has frequently been pointed out (Kleitman, 1929). The hypothalamus apparently plays a role in the discharge of these tensions.

Discussion

The constant alteration in brain temperature during sleep is a smoothing off of the jagged activity curve and a fall in hypothalamic temperature greater than elsewhere. Exceptions occur: the plateau may be broken due to restless sleep with much movement; and a preceding cold meal may have lowered brain temperature before sleep so much (1.0°) that it continues to rise even when sleep sets in. Still other irregularities occur uncommonly and are not explained.

The fall in temperature is not attributable to changed blood flow, as shown above. Gibbs (1935) also failed to find an altered flow in the human jugular during sleep. An increased heat loss from the brain through its coverings (Serota and Gerard, 1938) would affect cortex far more than deeper structures, which is not the case. The only satisfactory explanation of the cooling is a decreased heat production due to diminished neurone metabolism. The findings of Lampl and Feitelberg (1935) further indicate this. A thermocouple in the cat's cortex registered 0.5°C. warmer than that in the carotid artery during ordinary waking activity, 0.2° warmer during rest, and actually lower under paraldehyde anaesthesia.

The greater fall in hypothalamic than in other brain temperatures during sleep indicates a specific diminution of activity of these centers. This is in accord with the findings of Ranson (1934) and of Bard (1928), who found that stimulation of the hypothalamus leads to increased general activity, and indicates that sleep is associated with depression of the hypothalamus rather than its stimulation. The apparently opposed findings of Hess (1931) may perhaps be due to a depression rather than a stimulation of the "sleep center" by the sleep-producing slow currents used by him. Certainly the induction of sleep in his preparations by the injection of ergotoxin into the third ventricle is more easily explained by the view advanced above.

The technique of chronic temperature measurements of local brain regions obviously lends itself to many uses. For example, benzyl-methyl carbinamide (Benzedrine, 10 mg. subcutaneously) has been found (Serota and Schreider, unpublished) to increase temperature in all brain areas studied. The irregularities are supplanted by a smooth parabolic curve which rises and falls symmetrically over a period of four to eight hours, during which time the animal shows minimal increase in motor activity but marked decrease in threshold to faint light or sound stimuli. Serota and Gerard, in preliminary experiments,

have found no evidence of a differential depression of either cortex or thalamus during the anaesthetic action of ether, nembutal, etc , though all narcotics markedly lower brain temperature in relation to general body temperature

CONCLUSIONS

A technique is presented for following the temperatures of local brain regions in the unanaesthetized cat

In the conscious, as in the anaesthetized animal, basal brain regions are warmer than the cortex Absolute temperatures fluctuate during the waking state, sometimes in a semirhythmic manner, and the positive temperature difference between hypothalamus and cortex increases in irregular fashion with activity

Emotional states, as fear, rage, or anticipation of food, increase the relative temperature of the hypothalamus, sleep, especially, decreases and stabilizes it

On awakening from sleep, hypothalamic temperature rises earlier and further than that of the cortex, caudate nucleus, or Ammon's horn

The specific temperature decrease of the hypothalamus in sleep is shown to be due to lowered cell metabolism rather than to any marked change in blood flow This indicates that sleep is associated with a decreased rather than an increased activity of a hypothalamic "sleep center "

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FACTORS INFLUENCING BRAIN
POTENTIALS DURING SLEEP*

HELEN BLAKE, R. W. GERARD, AND N. KLEITMAN

From the Department of Physiology, University of Chicago

LOOMIS, HARVEY, AND HOBART (1937) have divided the period of sleep into several stages on the basis of changes in human brain potential patterns; Davis *et al.* (1938) have further analyzed the changes during the first part of the night; and Blake and Gerard (1937) have found delta wave intensity to parallel depth of sleep as determined by response to an auditory stimulus. Many bodily states show diurnal variations, for example: consciousness, movement, autonomic tone, skin resistance, and temperature (Kleitman, 1929). Some of these factors, as well as certain abnormalities in sleep, such as narcolepsy and sleep after experimental insomnia, have now been studied in relation to brain potentials in an attempt further to elucidate the mechanisms involved in the reversible change from wakefulness to sleep.

METHOD

Small silver (or solder) bipolar disc-electrodes, fastened to any two regions of the scalp (by collodion), were used to lead off the brain potentials in some experiments; more often monopolar leads were used consisting of a ring on the ear lobe and a disc on the vertex or occiput. Two independent, five-stage, resistance-capacity, push-pull amplifiers (time constant =0.5 sec.; Offner, 1936) fed cathode ray oscillographs and crystographs (Offner and Gerard, 1937).

Depth of sleep was measured as before by the duration of a constant sound required to elicit a response from the subject. Movement was measured by a motility box (Kleitman, 1932) and by muscle potentials picked up in the head leads. The latter was a more sensitive index, the record consisting of periods of muscle tension rather than of gross movements, and sometimes gave indication of changes not detected by the motility box. Temperature was taken orally. The subjects were usually placed on a bed in a darkened room, quiet except for the constant hum of a motor; but in one series of narcoleptics the subjects sat in an upright position and with ordinary illumination. Twenty-two experiments were performed on 8 normal adult subjects and 30 on 11 narcoleptics. One normal subject was studied during wakefulness and sleep after 100 hours of continuous experimental insomnia.

RESULTS

Factors in normal sleep

Comparison of leads. We have corroborated the findings of Loomis *et al.* (1937) and Davis *et al.* (1938) that during sleep the 10 per sec. trains are more often present at the occiput and the 14 per sec. rhythm at the vertex, and that the amplitude of the 1–3 per sec. waves is greater at the vertex. But, despite such detailed differences, all potentials are present over the entire cortex. When potentials from two head regions are compared, similar patterns of delta waves are clearly simultaneous at the parietal, occipital, frontal, and temporal regions, bursting into activity and subsiding suddenly (see also

Loomis et al., 1938). Further, whether or not the 10 per sec. rhythm is present
in all regions at a particular time, all leads may show bursts at this frequency
at some stages of sleep. With leads from only the occipital and frontal regions,
Blake and Gerard found the 14 per sec. rhythm inconspicuous; with the vertex
lead it is now clearly seen, even without specially tuned circuits. The fraction
of the time during which the 14 per sec. rhythm is present increases slowly,
and with fluctuations, during the early part of the night (alpha +delta period,
see below), reaches a maximum in the middle portion, and declines gradually
as the alpha waves again appear. The amplitude of the rhythm is lowest in
the period during which the slow waves predominate (compare Davis et al.,
1938).

Table 1. Potentials in stages of sleep

	Amt Alpha	Amt Delta	Amt 14 per sec	Depth of sleep	Present Nomenclature	Nomenclature of Tuxedo Park Group*
1	++	−	−	Awake	Alpha	A Interrupted alpha
2	+	++	+	Light sleep	Alpha +delta (+14 per sec.)	B Low voltage
						C Spindles
3	−	+++	+	Deep sleep	Delta (+14 per sec.)	D Spindles +random
						E. Random
4	−	−	+	Light sleep	Null (or low voltage)	B Low voltage
5	(+)	−	(+)	Sleep to wake	Intermittent alpha	B to A Low voltage to interrupted alpha
6	+	−	−	Awake	Alpha (low intensity)	

* The nomenclature of the Tuxedo Park group represented above is the result of personal communication with Dr. E. N. Harvey and Dr H Davis.

Sleep stages. Loomis et al. (1937, 1938), using tuned and untuned circuits,
have described five stages of sleep: A, alpha; B, low voltage; C, spindles; D,
spindles +random; and E, random. Potential patterns corresponding with
these stages have been observed in the present experiments but others are
also evident. The changes of potential pattern in the course of the night seem
best described in terms of the combined curves of rise and fall of each indi-
vidual rhythm. Despite the large and frequent fluctuations from one potential
pattern to another (Blake and Gerard, 1937; Loomis et al., 1937), especially
when the subject is disturbed, there is a definite slow shift through the night
in potential pattern prominence. This has been evaluated for the two faster
rhythms, as previously for the slow one, by averaging the dominant potentials
over 5 min. periods through many nights of sleep. Curves illustrating the
per cent presence of alpha, delta and 14 per sec. rhythms are shown in Fig. 1.
Fluctuations lasting 2 minutes or less (due to extraneous sound stimuli,
etc.) are not considered.

This set of curves permits a description of potential changes during the night. The major patterns are presented in Fig. 2. This arrangement does not conflict essentially with the stages of Loomis *et al.* (1937) and seems preferable since it is based upon the quantitative measure of per cent presence of each type of wave rather than upon the more qualitative recognition of certain potential patterns. For convenience, the generally accepted, although perhaps misleading, convention has been used of assigning a letter to each wave fre-

SCHEMA OF POTENTIALS DURING A NIGHT'S SLEEP
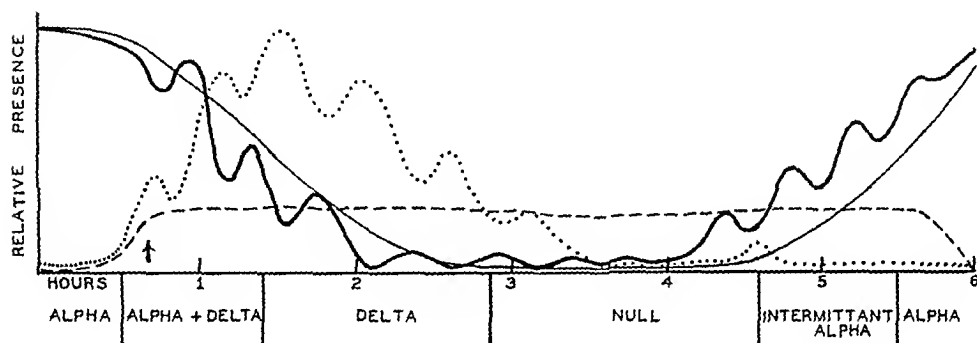
FIG. 1. Predominance of brain potentials through the night. Alpha waves (black heavy line) in per cent presence; 14 per sec. waves (dashes) in per cent presence and delta waves (dots) in extent of predominence. Oral temperature (black thin line). Below the stages of sleep are indicated. Record begun at time of retiring arrow indicates beginning of sleep. Wavy lines represent changes in waves due to shift of state of sleep. Depth of sleep by auditory response method roughly parallels delta curve.

quency. "Alpha" stands for the 10 per sec. regular rhythm usually present during the waking state. (In cases where the beta waves are the normal resting potentials, beta would replace alpha. "Alpha+delta" would be replaced similarly by "beta+delta.")* "Delta" is used for the 0.5 to 5 per sec. sometimes irregular wave dominant in deep sleep. The period of light sleep in the third quarter of the night, when delta waves have disappeared and the flat base line is broken only by an infrequent slow wave, or by a burst of alpha or beta waves, is called "null" (or low voltage) (Table 1).

The changes during diminishing sleep in the late part of the night fail to mirror those of increasing sleep in the early part in the following respects

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* Personal communications from Drs. Harvey and Davis have brought up the question whether the 10 per sec. rhythm seen simultaneously with the delta rhythm in the "alpha+delta" period is the same as the 10 per sec. wave of wakefulness or whether it is the beta component which has been slowed from 25 to 10 per sec. This question cannot be answered from the present work. It seems clear that the 10 per sec. rhythm in this "alpha +delta" period is not so closely associated with consciousness as it is later in the night (see below) and may, therefore, have a different significance. On the other hand, normal "beta" subjects show a "beta+delta" stage in contrast to the more common "alpha +delta"; and notched waves at this stage in the alpha case seem to be in transition between alpha and delta.

(Fig. 2): (i). The delta component reaches a peak in the second hour of sleep, then gradually disappears in another hour or two. (ii). The delta waves first appear before the alphas disappear, producing "notched" waves (Fig. 2b; also Blake and Gerard, 1937); whereas later the deltas fade some hours before the alpha waves return, leaving an essentially flat base line. (iii). The alpha waves are 20 to 40 per cent larger and the betas more prominent and of higher frequency just before falling asleep than just after awakening. (iv). Consciousness is associated with the presence of alpha waves in the "null" period but
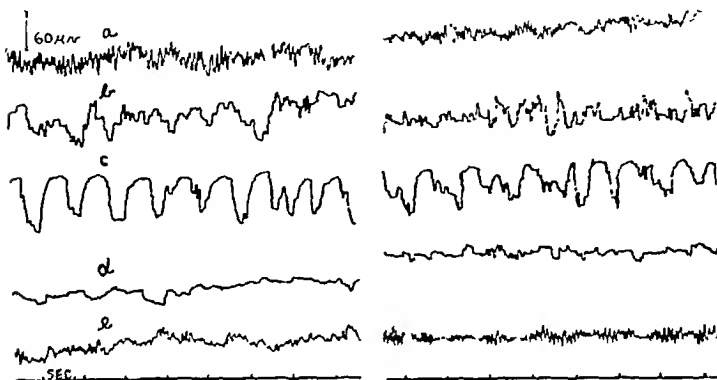
FIG. 2. Potential patterns through the night on three subjects. 1. "Alpha" rhythm of wakefulness. 2. "Alpha +delta" period of light sleep. 3. "Delta" period of deep sleep. 4. "Null" period of light sleep. 5. "Alpha" rhythm of wakefulness.

not necessarily in the earlier "alpha+delta" period. (v). Weak stimulation, such as slight movement or noise, affects brain potentials, particularly the delta rhythm, oppositely in the early and in the late period of light sleep (Loomis *et al.*, 1937; Blake and Gerard, 1937). In the "alpha+delta" stage it usually diminishes the delta waves, which emphasizes the alphas. In the "delta" and early in the "null" stages, a similar stimulus either exaggerates the slow waves already present or initiates them and also a faster rhythm, which last for a few seconds. This corresponds to the "K complex" of Loomis *et al.* (1938). Later in the "null" stage stimulation causes alpha waves to appear. It seems, then, that a stimulus which produces a shift towards lighter sleep causes the potentials present at the time to pass through those of the preceding stage before reaching the alpha waves of wakefulness.

Consciousness. The relation between consciousness and brain potentials was investigated by Davis *et al.* (1938) by having the subject squeeze a bulb when aware of having "drifted off." They found that alpha waves, which were absent during a "float," had returned between 3 and 23 sec. before the
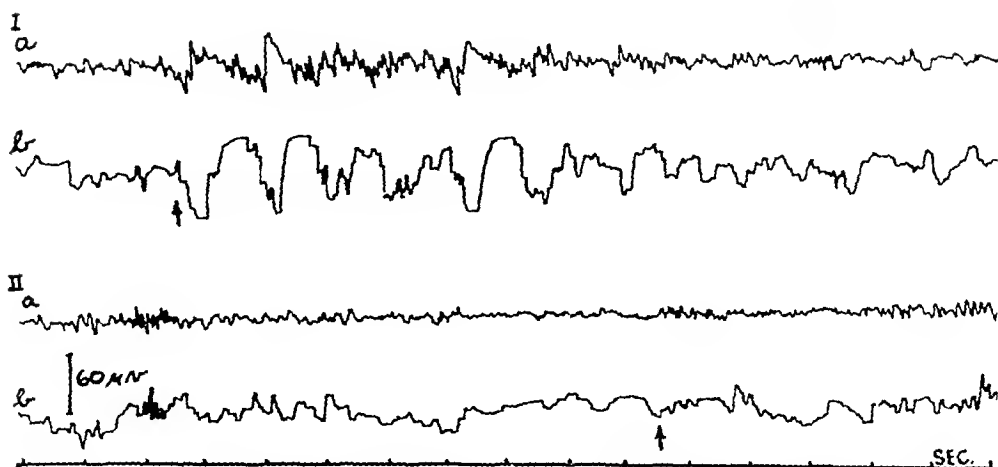
FIG. 3. Effect of slight stimulation (at arrows) on brain potential pattern: 1. As the delta waves are diminishing after deep sleep. a. Low cut-off filter in, b. No filter. 2. Late in the "null" period. a. Filtered. b. Unfiltered. Note that whereas in the first record stimulation elicits a train of delta waves, later it does not.

signal was given. We have studied the loss rather than the return of consciousness and related it to dreams and to skeletal muscle tonus.

A. *Dreams.* Loomis *et al.* (1936) at first suggested that dreams were associated with a "peculiar" slow wave, but later (1937) decided that they are not associated with any particular wave but occur in the B stage of sleep. Davis *et al.* (1938) find dreaming also in the C stage. In the present experiments, the subject was suddenly awakened while some particular wave pattern was present, and asked whether he had been asleep and, if so, whether and about what he had dreamed. Some individuals did not show sharp potential changes from one sleep level to another and others found it difficult to decide clearly whether or not sleep and dreams had been experience. In all subjects
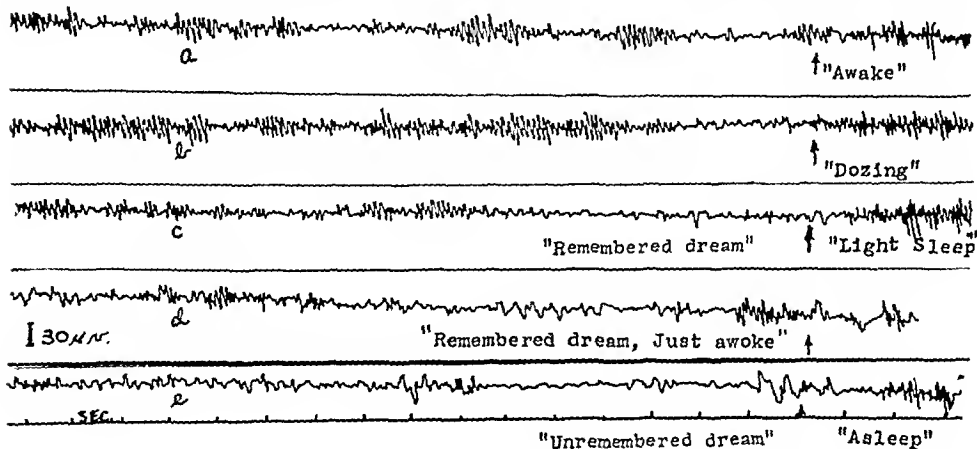
FIG. 4. Record in the "null" period of light sleep with low cut-off filter. Subject was awakened and questioned about dreaming at the arrow.

a uniform general correlation between potentials and dreams was present, but the following details are based largely upon results obtained on one young woman able to give decisive subjective reports (Table 2, Fig. 4): (i) Report, "awake." In the "null" period, the presence of alpha waves, even for one second, was invariably associated with a report of consciousness. (Fig. 4a); (ii) Report, "dozing"—some dimming of awareness and decreased sense of reality of surrounding events. This was the report when alpha waves had been absent for at least 3, average 6, sec. (Table 2). (iii) Report, "remembered

Table 2. *Brain potentials and introspection during sleep in second half of the night (one subject)*

Subjective Impressions	Duration of period with no alpha		Presence of delta	Number of queries
	Average sec.	*Range sec.*		
Wakefulness	0	—	–	6
Dozing	6	3–7	–	7
Remembered dream	9	2–16*	–	15
Unrecalled dream	55	6–120	+	6
Dreamless	?	?	+	Rarely occurred

* In one case there were no alpha waves for 150 sec. before the query but this is the exception; the usual figures are close to 9 sec.

dream"—light sleep with a dream clearly remembered. In nine-tenths of the trials, when alpha waves had been missing for 9 sec. (and no delta waves were present) the subject could remember a dream. (iv) Report, "unrecalled dream"—deeper sleep with a clear memory of having dreamed but no recall of content. This was the report when alpha waves had been absent, on the average, for 55 sec. Delta waves were usually present. (v) Report, "dreamless sleep"—deep sleep with no suggestion of having dreamed. This report paralleled a prominent delta rhythm, and rarely occurred in the second half of the night or early in the alpha+delta stage.

The subject was awakened at irregular intervals throughout the night in these tests and it is clear that dreaming was present most of the time, although minimal in the second quarter (delta period). The change from thinking to dreaming, with advancing sleep, seems to be less an immediate depression of mental activity than a progressive shift of attention from exteroceptive sensations towards subjective imagery. Even in deep "dreamless" sleep there is only a short period during which cortical activity is probably depressed to such an extent that the subject is not aware, on abrupt awakening, of having dreamed.

B. *Tonus.* As a test for tonus, the subject held between two fingers a light spool, which fell as the muscles relaxed in sleep. The subject was then aroused and asked whether or not he had been aware of dropping the object. The spool usually fell between 0.5 and 1.5 (average 1.1) sec. after the alpha rhythm had disappeared. The subject was then aware of its fall. Occasionally, however,

the fall was delayed until 6.5 to 25 (average 14) sec. after alpha loss, in which case the subject was unconscious of having dropped it. Tone, therefore, diminishes soon after the alpha rhythm is lost, but consciousness does not disappear for some seconds more. Subjective "dozing," shown above to follow the disappearance of alpha waves by 6 sec., is experienced between loss of tone and loss of consciousness. The subjects of Davis *et al.* (1938) similarly did not consider "floating" or "dozing" as real sleep.

Table 3. Effect of movement on brain potential patterns

Type of brain wave change	Duration of movement (sec.)	Duration of brain wave change (sec.)	Number of movements
$\alpha + \Delta \to \alpha$	21	14	6
$\Delta \to \alpha$	22	24	13
null $\to \alpha$	19	28*	20

* Several times changes, not included in the table, lasted for minutes and the level of sleep was permanently changed.

Motility. Blake and Gerard (1937) found that movement was regularly associated with a shift to lighter sleep. Loomis *et al.* (1937) report that "Movement may occur without a change of state (of sleep) and a change of state without movement, but frequently movement is immediately followed by a change of state upward, occasionally downward. . . ." In 90 per cent of movements in which muscle tension lasted over 5 sec. (80 instances now analyzed in detail) brain potentials shifted to a pattern of a lighter sleep; in 10 per cent they did not change, mainly in the null period. There was no shift towards a deeper level. (A transient increase in synchrony of delta waves in the third quarter of the night is evidence of decreased depth of sleep; cf. above.) Average values for the duration of movement (about 20 sec.) and the direction and duration of potential changes (progressively longer from the "alpha +delta" through the "null" stages) are given in Table 3. Occasionally sleep would remain lighter for over an hour following a movement. In some 5 per cent of all observations, alpha waves appeared before movement occurred, suggesting that extero- or interoceptive stimuli were responsible for the change; and even when brain and muscle activity were simultaneous such stimuli may have lightened sleep sufficiently to permit proprioceptive reflexes to break through. Certainly after movement is initiated the new proprioceptive barrage would tend to cause awakening.

Temperature. Alpha frequency varies with temperature in the waking subject (Hoagland, 1936; Jasper, 1936). The diurnal temperature change, 0.5°C., would only account for a frequency change between 10 and 9.7 per sec., assuming the mu value of 7000–8000 cal. (Hoagland). There is a 10–20 per cent slowing of the alpha rhythm with the onset of sleep (Davis *et al.* 1938) and a further diminution through the night, in close parallel with the amount of tremor (Jasper, 1938). We find the per cent presence of alphas parallels the temperature curve during sleep rather closely as both fall to,

and maintain, a low level, while only late in the "null" period does the alpha curve rise in advance of that for temperature (Fig 1) This correlation is reasonable since the alpha rhythm is associated closely with tonus (See above, also Jasper, 1938) A decline in muscle tone should decrease body temperature gradually, an increase in tone should raise temperature, but not so quickly as it restores the alpha rhythm Both the alpha rhythm presence and the temperature are lower in the morning after waking than they were the previous evening

Abnormal conditions

Experimental insomnia Behavior and potentials during prolonged insomnia and subsequent sleep have been studied (Kleitman, 1923, Blake and Gerard, 1937)
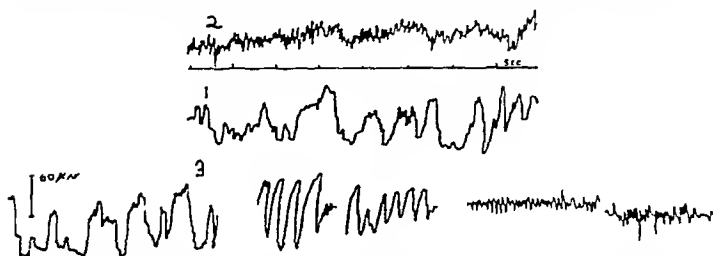
FIG 5 Record of subject with 100 hours' insomnia 1 "Normal" record 2 When subject was trying to concentrate on counting 3 Several regular potentials appearing within a 5 min period

Observations have now been made on one subject, of the dominant alpha type, after 100 hours of insomnia (Benzedrine was taken at intervals, Kleitman, unpublished) Slow waves predominated with the subject recumbent, even though talking or with the eyes open, and they disappeared only when he made an extreme effort to concentrate The 3–5 per sec rhythms with 14 per sec superimposed were the most common potentials, but all varieties of slow waves appeared and in no regular sequence with deepening sleep Muscular tone was so low that even with distinct effort the spool was never held more than 15 sec, and usually it was dropped immediately

The subject was never aware of having slept and thought he answered every question, but actually a strong auditory stimulus was often required to arouse him to the point of responding, which shows failure to differentiate sleep from wakefulness To check the maximum duration of wakefulness, the subject counted as long as he could This required intense concentration and was paralleled by a great discharge of beta waves which displaced the delta rhythm Even with this effort, consciousness was lost at a count between 3 and 10 and, in about as many seconds, the potentials drifted back to the usual slow ones Possibly most striking, was the play of many regular rhythms
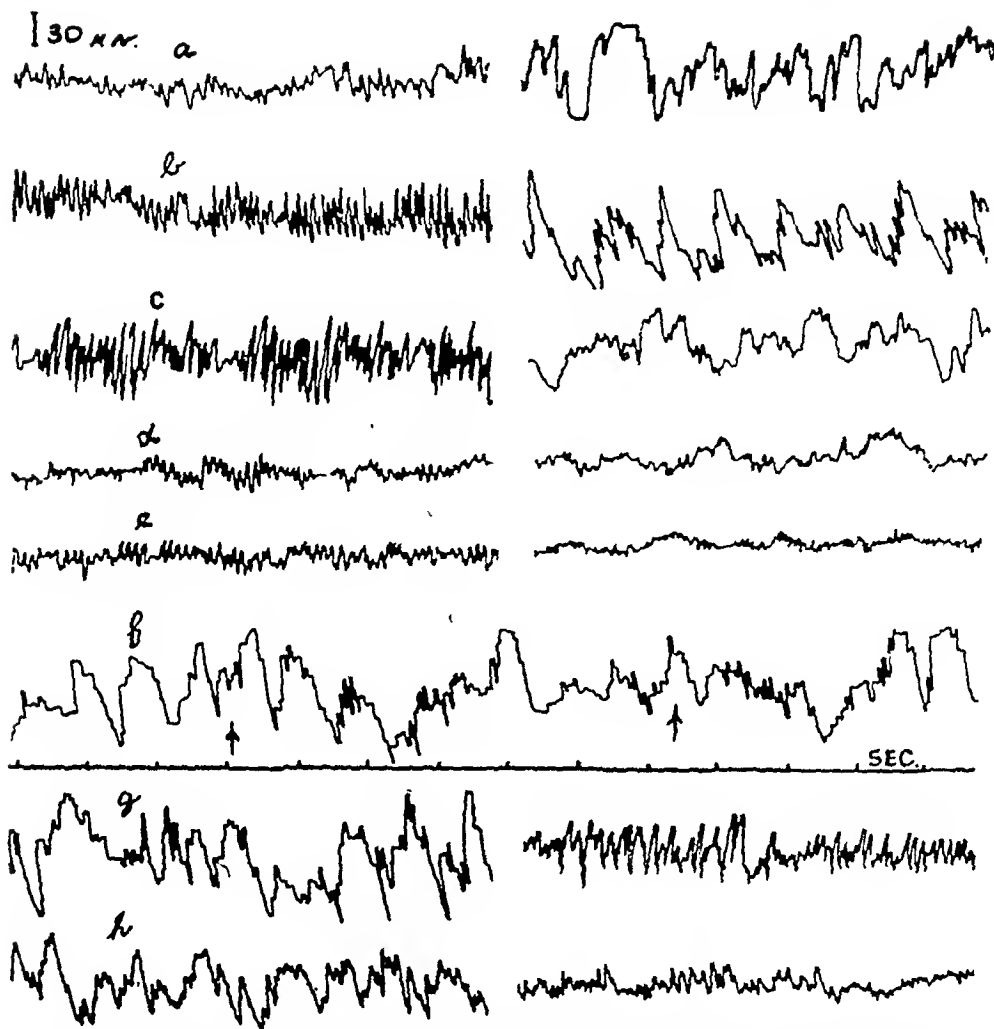
FIG. 6. a, b, c, d, e. Characteristic records of potentials from narcoleptics: 1. Lying down. 2. Sitting up. f. Response of narcoleptic to query in spite of presence of delta waves. g. h. Record of two narcoleptics lying down. Left. Before benzedrine. Right. One hour after 10 mg. benzedrine orally.

Within a five minute period: 1, 2, 3, 10 and 14 per sec. rhythms were clear, besides the high frequency beta discharge (Fig. 5). The genesis of these rhythms will be discussed later.

Narcolepsy. Eleven narcoleptic patients, male and female, of varying ages, were studied for four months during day and night sleep. Two had an associated obesity and one was an alcoholic.* In 10 of the cases a good alpha predominated in the sitting position; but on lying down this was always markedly diminished, and was usually replaced by large delta waves. The change oc-

* We are indebted to Dr. Walter Adams for the opportunity to study these patients. He will report elsewhere on the clinical aspects of this group of narcoleptics.

curred simultaneously in occipital, frontal, parietal and temporal leads, as in normal sleep. Delta frequencies from 0.5–5 per sec. appeared irregularly in each case (Fig. 6), much as in deep normal sleep. This is in contrast to the normal pattern of rest, in which the alpha rhythm persists through hours in the recumbent position, and even to that of day-time naps, in which the alpha rhythm is often merely depressed and delta waves are uncommon (Blake and Gerard, 1937). The correlation between depth of sleep, as determined by the auditory response method, and type of potentials was normal. Some patients characteristically slept deeply, others lightly, but all showed wide variations in sleep level. Since the changes between wakefulness and sleep are marked so clearly in these patients by changes in brain potentials, the electrical method may prove valuable for the objective determination of the frequency and duration of the narcoleptic attacks. It should be particularly useful in stuporous patients for discriminating sleep from simple unresponsiveness.

Drugs (a) *Benzedrine.* This drug is reported (Davidoff and Reifenstein, 1937) to increase wakefulness, excitation, mental activity, metabolism, and sympathetic stimulation, and was found (Blake and Gerard, 1937) to diminish the large delta waves present during sleep after prolonged insomnia. In 10 experiments, benzedrine sulphate (10 mg.) did not obviously affect the frequency or amplitude of the waking alpha rhythm, when psychological factors were controlled. During sleep, however, the delta potentials were diminished in duration and amplitude. This was particularly marked in narcoleptics (Fig. 6).

(b) *Alcohol.* Mullin *et al.* (1937) reported that alcohol increased depth of sleep during the first part of the night and decreased it during the second half. This has been confirmed (4 experiments) and a parallel change in potentials demonstrated, delta waves being accentuated early in the night, alpha waves later. Possibly the early peripheral vasodilatation, and consequent lowering of body temperature, is one factor favoring the early deep sleep.

DISCUSSION

If one defines sleep in terms of loss of consciousness (Kleitman, 1929; Hess, 1932), the light sleep of day-time naps offers a simple case for study of the essential changes. Here the constant alteration is a diminution of the alpha rhythm. Delta waves, or the 14 per sec. rhythm, may or may not appear. Loss of consciousness and of alpha rhythm are related and the alpha rhythm, further, is associated with muscle tone, the two decreasing together. Kleitman (1929) has emphasized the importance of diminution in proprioceptive and other afferent stimuli in inducing sleep and suspending consciousness, so the close relation of alpha waves to both tonus and awareness is significant.

The work of Bremer (1935, 1937) likewise emphasizes the importance of diminished afferent impulses in producing the cortical potential changes of sleep. In the cat, mesencephalic section of the brain stem, barbiturate narcosis, and sleep are all associated with cortical waves of decreased frequency

and increased amplitude. Several workers (Jasper, 1937; Gerard, 1936; Blake and Gerard, 1937) have emphasized the relation of neural excitation level and wave frequency, the two rising and falling together. The present findings with a stimulant, benzedrine, and a depressant, alcohol, fit this picture. On this basis, diminished afferent impulses (perhaps most important, the proprioceptive) playing upon the brain, allow cortical excitation to subside with the gradual loss of consciousness and slowing of potentials. The beta waves retard to 14 per sec. spindles, as Jasper (1937) has shown; and the alpha waves are replaced by, or perhaps are changed into, the slow deltas (Blake and Gerard, 1937). Certainly the appearance of many distinct frequencies within a few minutes, after a prolonged insomnia, suggests that the same cortical neurones can beat at many rates; and the demonstration (Libet and Gerard, 1938 and unpublished) that a few homogeneous cells in the isolated frog olfactory bulb can be made to assume regular rhythms from 1 to 50 per sec. by controlling the excitation level, strongly supports such an interpretation.

It remains uncertain whether the slowed cortical rhythms of sleep are a direct consequence of lowered afferent bombardment or are secondary to a decreased cell metabolism which follows the lowered excitation level. Certainly sensory impulses increase brain heat production (Serota and Gerard, 1938) and, conversely, in sleep brain temperature falls (Serota, 1939). It is also not clear what the role of subcortical centers may be. The fairly simultaneous change in waves over most of the cortex would be in accord with a thalamic or hypothalamic control; and much evidence for such "sleep" centers exists (Hess, 1932; Ranson, 1934; Bard, 1928; Serota, 1939). The cortical changes could, of course, result from a shutting of the thalamic gateway to sensory impulses as well as from a failure to initiate them at peripheral receptors. The excessive somnolence of narcolepsy, associated with pathology in the diencephalon, may well depend on such a "blockade"; that following prolonged insomnia is more probably compounded from both these factors and a direct fatigue depression of cortical neurones as well.

Interpretations of the findings concerned with dreaming is partly beyond the scope of this paper, especially in view of the vast psychiatric literature dealing with the dynamic properties of dreams. We have shown that a subject abruptly awakened, almost at any time during the night, can recall having dreamed; the longer the immediately preceding period with no alpha waves, the less is the recall, and when this period is about a minute (especially if delta waves are present), there is no trace of a dream's having been in progress. This largely excludes the possibility that, in the other states, the dream ran its course as a flash while the subject was actually in the process of waking; presumably dream consciousness, like waking consciousness, blurs and fades progressively as the activity of the cortical neurones falls to lower and lower levels. The conclusion that delta waves appear only in complete unconsciousness, such as coma, narcosis, and epilepsy, as well as dreamless sleep, is forbidden by their presence in drowsing narcoleptics.

Finally, the sequence of changes in passing from wakefulness to deep sleep

and back to wakefulness is of interest. Muscle tone decreases first, then sharp awareness is replaced by dozing or actual dreaming but the subject can still take cognizance of events (dropping a spool, having dreamed), and finally a dreamless oblivion of deep sleep is reached. Disturbances during sleep—external or proprioceptive or perhaps even the building up of excitation in the brain itself—cause a shift towards a lighter state. This is more prolonged when the disturbance occurs late in the night than when it is early and so parallels other signs of asymmetry during a night's sleep. Thus: delta waves appear before the alphas are gone during deepening sleep, but disappear before the alpha waves return during the "null" period; in the descending phase, stimuli convert delta waves to alphas, while in the ascending stage they first initiate delta waves and start alphas only if actually arousing the sleeper; and the alphas just after awakening are feebler than just before going to sleep.

This asymmetry could be accounted for by a combination of two factors. The "fatigued" cortical cells easily fall to a low level of activity when afferent impulses decrease. As they become "rested," sleep lightens even without increased stimulation; and any stimuli that do occur then are relatively more effective than earlier ones.

SUMMARY

1. Minute to minute fluctuations in brain potentials through the night are superimposed on a gradual trend from hour to hour. This latter is compared with the sleep stages described by others. The potentials are present simultaneously over much of the cortex. In sequence, the patterns are: alpha + delta, delta, null, intermittent alpha.

2. During increasing sleep depth, early in the night, delta waves appear before alpha waves are gone; while later, during diminishing depth of sleep, the deltas disappear before the alphas return. In other respects also, potentials of the rising sleep phase do not mirror those of the falling phase.

3. Subjective reports of sleep and dreams can be correlated with potential patterns, sometimes quite sharply.

4. Movement is accompanied by a shift of potentials towards lighter sleep in nine-tenths of the present cases, by no change the remaining times.

5. Hypersomnia, due to prolonged voluntary insomnia or to narcolepsy, is associated with delta waves at relatively higher levels of consciousness than in normal sleep.

6. A stimulant drug, benzedrine, diminishes delta waves; a depressant, alcohol, enhances them.

7. These findings are discussed in relation to theories of sleep and the source of cortical potentials.

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HYPOTHALAMIC REGULATION OF
BODY TEMPERATURE*

GEORGE CLARK, H. W. MAGOUN, AND S. W. RANSON

Institute of Neurology, Northwestern University Medical School, Chicago

IT HAS RECENTLY been shown that cats with lesions in the suprachiasmatic region and anterior part of the hypothalamus become overheated when exposed to external temperatures of 102 or 104°F, and that they do not react as readily as normal cats by increased respiratory rate and panting (Teague and Ranson, 1936). In monkeys lesions in the rostral part of the lateral hypothalamus tend to cause a transient hyperthermia while lesions in the caudal part of the lateral hypothalamus cause prolonged hypothermia (Ranson, Fisher and Ingram, 1937). References to other investigations of temperature regulation will be found in the papers listed at the end of this article.

The present paper presents the results of a reinvestigation of the relation of the hypothalamus to temperature regulation. In some cats lesions were placed 1 mm. to the right and left of the midline at the level of the chiasma and in the midline at the level of the infundibulum, causing medially placed damage. In some the lesions were placed at or immediately behind the level of the chiasma and 3 mm. to the right and left of the midline, causing laterally placed damage. In others lesions were placed lateral to the mammillary bodies. In one animal a medially placed lesion destroyed both mammillary bodies. In these animals the lesions were of moderate size. In other experiments huge lesions were made. In some of these the damage was anteriorly placed, in others it was in the middle of the hypothalamus and in still others in its caudal part.

METHODS

The lesions were made with the Horsley-Clarke instrument in the manner previously described (Ingram and Ranson, 1932) except that the two poles of the bipolar electrode were separated by 2 mm along the long axis of the electrode in order to produce a cylindrical lesion with its long axis corresponding with that of the electrode.

Preceding the operation, daily observations were made of the cat's rectal temperature for a week or more and tests were made of the ability of the animal to regulate its body temperature in the cold box and in the hot box. After the operation the cats were kept for one or more days in an incubator set to run at 86° but varying between 78 and 90°F. Daily observations were made of the rectal and environmental temperature for 2 weeks or more. Tests were made in the hot and cold box about a week after the operation and repeated on the second and fourth weeks and in some cases after much longer intervals. The cold box was the same as that used by Teague and Ranson (1936) and its temperature ranged from 34° to 46° but was usually around 44°F. The hot box was altered by inserting a fan which allowed more even regulation of the temperature within it but because of the increased movement of the air, favored the evaporation of perspiration. The temperature in the hot box was 103 or 104°F. The cats were kept in the box until they panted or until the rectal temperature reached 106°.

FIG 1 The lesions in Cat 53 indicated in solid black on four drawings from transverse sections through the brain at the level of and in front of the anterior commissure, lettered in order from before backward

ABBREVIATIONS FOR ALL FIGURES

A,BC,D,	Order of sections caudalward
AC	Anterior commissure
CP	Cerebral peduncle
E	Entopeduncular nucleus
F	Fornix
H	Field H of Forel
HG	Habenular ganglion
HP	Habenulopeduncular tract
Hy	Hypophysis
IC	Internal capsule
LV	Lateral ventricle
MLF	Medial longitudinal fasciculus
ME	Median eminence
M	Mammillary body
MP	Mammillary peduncle
MT	Mammillothalamic tract
NII	Optic nerve
NIII	Third nerve
OC	Optic chiasma
OT	Optic tract
P	Globus pallidus
PC	Posterior commissure
S	Septum
Sth	Subthalamic nucleus
SN	Substantia nigra
V3	Third ventricle
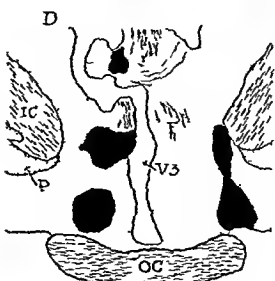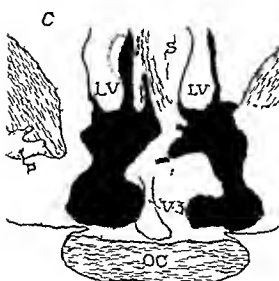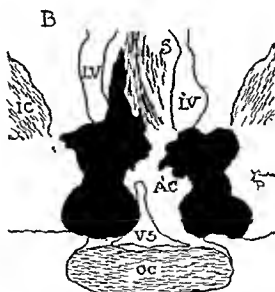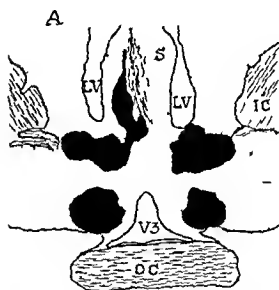
FIG 2 The lesions in Cat 51 indicated in solid black on four drawings from transverse sections through the brain at the level of and behind the anterior commissure.

RESULTS

In order to form a basis for comparison it was necessary to determine the range of variation in the temperature of normal cats. It can be stated on the basis of 384 observations that the average normal rectal temperature of the cat is 101.4°F. But there is a considerable range of variation so that neither 99.5 nor 102.9° can be considered abnormal. Based on 90 tests, the average rectal temperature at which normal cats begin to pant in the hot box is 103.2° with variations between 105.4 and 101.4°. In the 90 tests, the panting level was above 104.5° in 11, and below 102° in 12. After three hours in the cold box the normal cat's temperature may either have risen or fallen a trifle or if the temperature was high to begin with falls of 1.0 or even 1.5 may occur. A fall of more than 1.0 to a final temperature below 100° is suspicious and any fall below 99.5° is abnormal. Large lesions in the hypothalamus resulted in the death of most of the animals. They would not eat spontaneously and most of them had to be fed by tube as long as they lived. Diarrhea was not uncommon and the nutritive condition poor. The resistance of the animals to infection was low.

Large lesions of the anterior group (Fig. 1 and 2) were less fatal than those situated farther caudally and within this group those situated at the level of or in front of the anterior commissure (A.C. and A.A.C., Table 1) were less fatal than those in the anterior hypothalamus (A.H., Table 1). Of the 20 cats in the anterior group only 8 survived for 1 month or more and only 2 of these were among the 11 with lesions in the anterior hypothalamus. Like all the others these 8 required tube feeding but 6 ultimately ate spontaneously and these were the only ones that survived more than 5 weeks. The night following the operation about half of the animals with anterior lesions defecated excessively, passing large amounts of liquid or soft stools. In these cats insertion of the thermometer for rectal temperature usually caused during the next few days violent defecatory movements and expulsion of the thermometer unless it was firmly held in place. Some of these cats had a diarrhea for several days following the operation. These symptoms were not seen in the two cats with lesions in front of the anterior commissure. Considering their poor physical condition the cats were reasonably alert and showed good motor initiative. None showed catalepsy. One animal showed sham rage and 5 others were extremely surly.

When removed from the incubator on the first morning after the operation the 2 cats with lesions in front of the anterior commissure both had rectal temperatures above 104.5°; 4 of the 7 with lesions at the level of the anterior commissure had temperatures of 104.5° or higher and the lowest temperature in this group was 99°; 3 of the 11 with lesions in the anterior hypothalamus had temperatures above 104.5° and 4 had temperatures below 98°. One of these (Cat 5) continued to run a subnormal temperature and was dead on the fifth day. The temperature of the other 3 was normal or above normal on the second and third days in the incubator. While a majority of the cats of this anterior group ran temperatures which were normal or slightly above on the

Table 1. Postoperative rectal temperatures of cats with very large lesions. The figures in parentheses indicate the temperature of the room or incubator. The cats are listed in three groups according as the lesions are placed in the anterior, middle or posterior parts of the hypothalamus, those of the anterior group are again divided into three divisions designated by the letters A.A.C., cats with lesions anterior to the anterior commissure; A.C., cats with lesions at the level of the anterior commissure; A.H., cats with lesions in the anterior hypothalamus behind the anterior commissure. The cats of the middle group are subdivided into those designated by letter I. with lesions at the level of the infundibulum and those with lesions extending from the level of the infundibulum to the mammillary bodies (I.M.).

Cat	1st Day	3rd Day	5th Day	7th Day	10th Day
Anterior					
A.A.C.					
53	104.6 (85)	104.9 (77)	102.5 (75)	102.6 (75)	102.4 (76)
54	105.6 (85)	103.1 (77)	105.2 (75)	106.0 (75)	Dead
A.C.					
51	99.4 (82)	102.4 (77)	102.0 (75)	98.6 (75)	99.8 (75)
52	105.1 (85)	103.2 (77)	103.7 (75)	103.1 (75)	102.9 (76)
92	105.6 (81)	101.9 (72)	100.4 (73)	101.0 (72)	100.3 (74)
93	99.0 (81)	100.0 (81)	97.7 (81)	<94.0 (81)	Dead
94	104.9 (80)	102.6 (81)	104.4 (81)	99.4 (74)	96.4 (72)
96	104.5 (80)	102.7 (71)	103.3 (73)	101.8 (74)	100.2 (72)
97	100.6 (81)	100.6 (71)	102.0 (72)	100.3 (76)	98.2 (75)
A.H.					
2	98.9 (90)	101.1 (85)	Dead		
5	94.2 (90)	94.9 (84)	Dead		
12	98.3 (84)	97.7 (74)	98.7 (76)	103.6 (76)	97.6 (75)
26	108.5 (89)	98.5 (82)	Dead		
27	105.0 (80)	103.6 (79)	102.9 (77)	102.2 (79)	Dead
79	103.4 (84)	103.6 (75)	100.0 (73)	95.3 (73)	96.9 (74)
80	104.1 (81)	98.3 (71)	94.9 (73)	Dead	
81	105.3 (81)	103.2 (71)	99.2 (73)	99.6 (73)	100.6 (72)
82	95.8 (84)	101.5 (80)	94.0 (73)	94.4 (75)	92.7 (72)
83	94.3 (80)	104.2 (80)	101.4 (75)	96.3 (72)	98.2 (73)
85	96.9 (84)	107.9 (73)	105.9 (74)	Dead	
Middle I.					
32	94.4 (84)	92.0 (83)	92.2 (84)	98.4 (88)	Dead
33	96.2 (82)	92.3 (77)	100.8 (85)	93.5 (75)	Dead
34	96.4 (86)	96.8 (85)	93.3 (75)	95.8 (84)	Dead
36	99.1 (88)	99.8 (74)	102.3 (75)	101.1 (75)	Dead
37	102.4 (88)	96.3 (74)	95.3 (75)	Dead	
I.M.					
31	96.6 (79)	93.6 (82)	99.5 (86)	98.4 (75)	100.3 (77)
35	100.3 (86)	93.1 (85)	101.3 (75)	94.4 (75)	Dead
Posterior					
28	95.2 (85)	96.6 (84)	103.1 (84)	100.0 (84)	Dead
71	92.5 (83)	103.4 (83)	Dead		
72	94.4 (83)	Dead			
73	93.4 (84)	100.9 (82)	Dead		
74	<92.0 (84)	98.2 (82)	Dead		

second and third days some of these developed subnormal temperatures be
fore the tenth day (Table 1) It is important to note that the incidence of
subnormal temperatures increased with the lapse of time following operation,
which is just the opposite of what is seen after lesions farther back in the
hypothalamus Cat 97, whose temperature was normal for the first 5 days and
varied from normal to slightly subnormal during the next 15 days, was found
on the 60th day, following an interval in which no temperatures were recorded,
to have a rectal temperature of 97° During the next 9 days its temperature
did not rise above 98° It was below 97° on 6 of the 9 days and once reached
94 1° This animal was in excellent physical condition and had a good appetite
and a sleek coat of hair

Because of the early death or poor condition of a majority of these cats
only 7 were tested in the cold box and 8 in the hot box Three of the 7 showed
abnormal reactions in the cold box, their rectal temperature dropping to
92 4, 95 and 95 4° respectively Two of these 3 shivered and in the third shiver
ing was questionable (Table 2)

*Table 2 Cold box tests on cats with large anteriorly placed lesions Rectal
temperature in degrees Fahrenheit*

Cat no	Days after op eration	Level of lesion	Temp at start	Temp at end	Change	Shiver ing	Av temp of box
53	14	A A C	102 0	101 9	−0 1	?	49
51	15	A C	103 1	102 7	−0 4	?	49
94	18	A C	98 0	95 4	−2 6	yes	42
96	79	A C	99 1	95 0	−4 1	?	44
97	74	A C	98 0	92 4	−5 6	yes?	44
12	104	A H	101 2	101 0	−0 2	?	39
81	73	A H	103 6	101 6	−2 0	yes	39 5

Hot box tests showed that in this group of animals there was a marked
loss in ability to regulate against heat (Table 3) In all but one cat the rectal
temperature rose above 106° without causing panting or much increase in
respiratory rate In 6 of the cats the rate did not exceed 36 per minute One
cat (53) panted at a rectal temperature of 104 5° and it is interesting to note
that this was the only one in which the lesions were situated so far forward
as to spare most of the anterior commissure and much of the preoptic region
(Fig 1) In Cat 51 the lesions involved the anterior commissure and the brain
ventral to it thus largely destroying the preoptic region (Fig 2) In the hot
box test made on this cat 38 days after the operation the rectal temperature
reached 106 4° without causing panting or an increase in respiratory rate
above 72 (Table 3) The difference between the reactions of these two cats
in the hot box, the one with the preoptic region largely intact showing a nor-
mal reaction and the one with this region largely destroyed showing a failure
to pant, fits very well with the results obtained by Magoun, Harrison, Bro-
beck and Ranson (1938) by local heating of the brain The anterior limits of
the area concerned with heat loss activity as determined by heating the brain

coincide within half a millimeter with the limits of this area as determined by lesions.

Large lesions of the middle group, destroying most of the hypothalamus from the chiasma to the caudal border of the attachment of the hypophyseal stalk (I., Table 1) and in two cases involving also the level of the mammillary bodies (I.M., Table 1), were invariably fatal. The period of survival of the 7 cats of this group varied from 5 to 11 days, the average being 8 days. None of the cats ate spontaneously. Two (31 and 35) with lesions extending far enough back to involve the level of the mammillary bodies were drowsy, cataleptic and lacking in motor initiative on the day following the operation, four of

Table 3. *Hot box tests on cats with large anteriorly placed lesions. Rectal temperature in degrees Fahrenheit*

Cat no.	Days after operation	Level of lesion	Temp. at end of test	Rise in temp.	Final respiratory rate	Panting	Sweating
53	35	A.A.C.	104.5	3.6	140	yes	yes
51	38	A.C.	106.4	4.3	72	no	not tested
94	30	A.C.	106.2	13.8	16	no	no
96	81	A.C.	106.4	8.3	22	no	no
97	64	A.C.	106.2	7.8	24	no	not noticed
12	104	A.H.	106.3	4.3	36	no	no
81	71	A.H.	106.0	3.2	24	no	no
83	28	A.H.	106.2	5.6	32	no	no

the others were alert and active and the remaining one slightly less active. Only one of the cats defecated excessively. Two others made violent movements of defecation when the thermometer was inserted and another pair developed a marked diarrhea. Not one was excessively irritable. None of these cats shivered spontaneously. On several occasions one (32) was observed panting in the incubator, although it was running a rectal temperature of 94° or less. These cats ran for the most part subnormal temperatures although in three (35, 36, 37) the temperature was normal on the morning after the operation when the cat was removed from the incubator.

Hot box tests were made on 6 cats of this group. Five of them failed to pant before their rectal temperature reached 106°. The other cat (32), however, panted at 103.7°. This was the cat which on several occasions was observed panting in the incubator with a rectal temperature of about 94°. No cold box tests were made in this group.

Large lesions destroying the posterior part of the hypothalamus were made in 5 cats. None of these animals lived more than 7 days and the brains were not prepared for microscopical study; but free hand sections indicated practically complete destruction of the hypothalamus at the level of the mammillary bodies. The first morning after the operation the animals seemed asleep, the eyes were closed and they could not stand. Three were limp with no tone in the postural muscles. Two showed some tonicity on the first day but were

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flaccid the next day. As long as they lived these animals were absolutely quiet, remaining where and as they were placed. Due to the lack of muscular tonus they could not be posed in any position requiring muscular effort. None of these animals ever ate spontaneously and all required tube feeding. Two developed diarrhea. On the morning after the operation the highest temperature recorded was 95.2° and the lowest was below 92, Table 1. These cats ran subnormal temperatures in the incubator as long as they lived, except cats 28 and 73 which recorded 100° and 100.9° the day before they died and
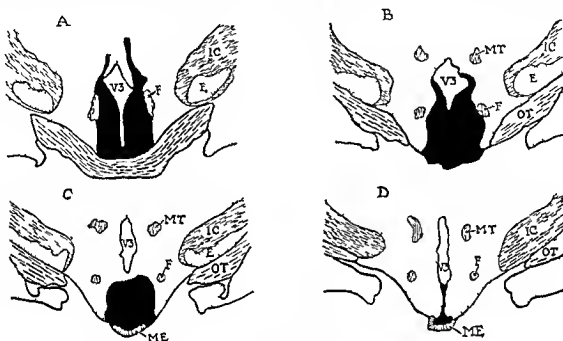
Fig 3 The lesions in Cat 13 indicated in solid black on four drawings from transverse sections through the brain at the level of and behind the optic chiasma

Cat 71 which had temperatures of 101.4 and 103.4 on the second and third days On autopsy the lungs were found congested or consolidated in each case. No hot or cold box tests were made on these animals.

Moderate sized lesions offer much more information about functional localization than do the massive lesions considered in the preceding section. These smaller lesions have been so placed as to destroy selectively rostromedial, rostrolateral, caudomedial or caudolateral portions of the hypothalamus.

Small or moderate sized lesions in the medial part of the anterior hypothalamus in 17 cats produced no easily recognizable symptoms On the morning after the operation these animals were alert and active. They usually ate spontaneously on the first or second postoperative day and none of them showed a postoperative diarrhea. Their daily temperatures subsequent to the operation were normal except that a considerable number of them had temperatures in the range of 104 or 105° on the first postoperative day. Eight of these cats on which hot and cold box tests were made are listed in Tables 4, 5 and 6. Their reactions in the cold box were entirely normal. In the hot box tests made one month after the operation their records were only slightly abnormal as shown by comparison with the similar preoperative tests recorded in the

same table. There was some elevation of the panting temperature level and decrease in final respiratory rate as judged by the preoperative controls; but they all panted at rectal temperatures below 106.°

The lesions lay close to the midline and in Cat 13 extended from the optic

Table 4. Postoperative rectal temperatures in degrees Fahrenheit of cats with moderate sized hypothalamic lesions. The figures in parentheses indicate the temperature of the room or incubator. The cats are listed in four groups: those with anteromedial lesions are designated by the letters AM; those with anterolateral lesions, AL; those with posteromedial lesions, PM; and those with posterolateral lesions, PL.

Cat	1st Day	3rd Day	5th Day	7th Day	10th Day
A.M.					
6	103.2 (84)	101.7 (75)	102.0 (75)	101.2 (77)	101.0 (77)
11	105.0 (84)	103.0 (75)	103.3 (76)	101.9 (76)	103.0 (75)
13	102.6 (84)	102.5 (76)	103.0 (76)	102.3 (75)	103.2 (75)
15	103.0 (87)	103.2 (76)	104.0 (75)	103.8 (75)	
16	105.4 (87)	104.9 (76)	102.9 (75)	103.3 (75)	
17	103.5 (87)	103.6 (75)	104.1 (75)	D.C.	
18	104.6 (87)	102.0 (75)	101.8 (75)	D.C.	
22	104.4 (82)	102.2 (74)	101.3 (75)	101.9 (74)	101.6 (73)
A.L.					
8	107.9 (84)	103.7 (76)	103.5 (77)	102.1 (74)	101.8 (76)
20	94.0 (78)	102.1 (85)	101.0 (76)	99.3 (76)	100.7 (75)
40	104.6 (82)	106.0 (74)	98.2 (74)	104.9 (72)	100.1 (74)
23	104.6 (85)	104.7 (76)	103.6 (76)	103.2 (75)	102.5 (75)
39	102.1 (82)	101.9 (74)	102.7 (74)	102.0 (72)	100.7 (74)
42	105.1 (85)	103.8 (76)	102.7 (76)	101.3 (75)	102.5 (75)
43	105.2 (82)	99.6 (74)	98.7 (74)	97.3 (72)	101.3 (74)
55	106.0 (82)	100.1 (76)	101.7 (76)	102.4 (76)	
P.M.					
103	102.1 (86)	103.6 (76)	102.1 (76)	102.2 (76)	103.3 (74)
25	101.6 (81)	104.0 (77)	103.2 (75)	101.2 (75)	101.4 (75)
38	101.8 (81)	103.5 (77)	103.5 (75)	103.8 (75)	103.5 (75)
105	97.1 (84)	103.1 (77)	103.8 (77)	102.5 (77)	103.2 (77)
P.L.					
46	90.6 (83)	102.9 (82)	97.6 (74)	99.6 (74)	99.3 (77)
47	87.0 (83)	105.4 (82)	97.6 (74)	102.6 (74)	103.7 (77)
100	91.0 (82)	100.2 (?)	98.8 (77)	105.7 (79)	102.6 (77)
107	94.7 (82)	97.1 (79)	103.6 (77)	102.9 (75)	102.5 (75)
109	93.4 (88)	99.1 (90)	101.8 (91)	96.4 (73)	98.2 (74)
110	95.6 (84)	99.4 (86)	99.5 (84)	99.5 (76)	101.1 (74)
114	96.0 (85)	98.6 (84)	99.9 (73)	102.3 (75)	101.4 (77)

chiasma to the fornix and caudalward over the median eminence (the expanded end of the infundibulum where this is attached to the tuber) as shown in Fig. 3. In this cat the daily temperature remained within normal limits (Table 4). In some of the others temperatures of 104 and 105° were encountered. The important thing is that at no time did any of these cats show a loss in capacity to keep the body temperature up to the normal level. Five days after the

operation Cat 13 was in the cold box for 3 hours at a temperature of 42°
without any significant drop in its rectal temperature (Table 5).

When tested in the hot box one month after the operation Cat 13 panted
at a rate of 132 per min. at a rectal temperature of 104.1 as compared with
a respiratory rate of 230 per min. and a temperature of 101.7° in the pre-
operative test (Table 6). In hot box tests made one week after the operation
two of these cats with medial lesions failed to pant at temperatures below
106°. Of these two, one had a respiratory rate of 78 at 106.1° and the other,
a rate of 212 at 106.2°. At the end of a month, however, both of these cats
(11 and 22) had merely an increase in panting level of about 1.5° above the
preoperative level (Table 6). The cats in this group showed somewhat less
disturbances in regulation against heat than did those reported by Teague
and Ranson (1936) and this is to be correlated with the fact that the lesions
did not extend quite as far lateralward (Fig. 3). All of the cats of this group

*Table 5 Cold box tests on cats with medial lesions in the rostral part of the hypothalamus.
Rectal temperature in degrees Fahrenheit.*

	Preoperative tests					Tests made during the first postoperative week				
Cat no	Temp at start	Temp. at end	Change	Shiv-ering	Av temp of box	Temp at start	Temp. at end	Change	Shiv-ering	Av temp of box
6	100 8	100 8	0	yes	41	102 5	102 1	−0 4	yes	44
11	101 8	101 9	+0 1	yes	46	103 3	103 8	+0 5	yes	44
13	101 7	101 2	−0 5	yes	45	101 6	101 0	−0 6	yes?	42
15	101 3	101 0	−0 3	yes	45	102 4	102 1	−0 3	yes	42
16	101 3	99 6	−1 7	yes	43	104 5	103 6	−0 9	yes	42
17	102 5	101 1	−1 4	yes	46	102 9	102 1	−0 8	yes	43
18	101 7	101 7	0	yes	45	101 5	101 2	−0 3	yes	43
22	101 0	99 5	−1 5	yes	44	101 8	100 0	−1 8	yes	45

reacted normally in the cold box tests made during the first postoperative
week (Table 5).

Moderate sized lesions in the lateral part of the anterior hypothalamus
caused much greater disturbance in temperature regulation than did those
more medially placed. There were 8 cats in this series and of these, 3 (8, 20,
40) had symmetrically placed lesions in the extreme lateral part of the hypo-
thalamus, reaching and often damaging the medial edge of the basis pedunculi
and internal capsule (Fig. 4). The region, between the fornix and internal
capsule above the optic chiasma, which contains the medial forebrain bundle
was destroyed on both sides of the brain. The remaining 5 cats had lesions
asymmetrically placed so that a considerable part of the medial forebrain
bundle escaped damage on one or the other sides.

On the morning after the operation all 8 of these animals were alert and
active. Some of them had multiple soft stools during the night following the
operation. Two of the three cats with symmetrical lesions did not eat spon-
taneously after the operation but required tube feeding for the rest of their

lives. The other cats required tube feeding for a time which averaged about 11 days. In all but one case the rectal temperature was normal or above normal on the morning following the operation. The exception, Cat 20, had a subnormal temperature on the first postoperative morning but on the second and third days it was normal (Table 4). Six cats on the morning following the operation had temperature above 104.5 and in one it reached 107.9°. The
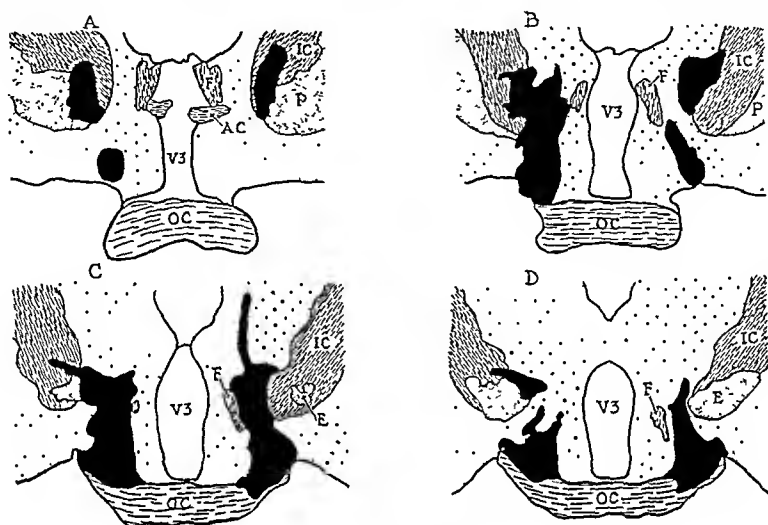
FIG. 4. The lesions in Cat 8 indicated in solid black on four drawings from transverse sections through the brain at the level of the optic chiasma.

second day three had temperatures above 104.5. Soon thereafter all temperatures became normal and remained so except for Cat 20 which for 23 days (from the 22nd to the 45th postoperative day) ran a subnormal temperature as low as 97°F.

Table 6. Hot box tests on cats with medial lesions in the rostral part of the hypothalamus. Rectal temperature in degrees Fahrenheit.

	Preoperative tests					Tests one month postoperative				
Cat no.	Temp. at end of test	Rise in temp.	Final respiratory rate	Panting	Sweating	Temp. at end of test	Rise in temp.	Final respiratory rate	Panting	Sweating
6	102.7	0.3	184	yes	no	103.0	2.2	146	yes	no
11	104.1	2.4	112	yes		105.7	4.2	186	yes	yes
13	101.7	0.9	230	yes	no	104.1	2.9	132	yes	no
15	103.0	0.5	160	yes	yes	105.9	3.5	126	yes	no
16	105.2	2.5	224	yes	no	104.1	2.0	196	yes	yes
17	102.1	0.8	216	yes	yes	105.3	4.0	180	yes	no
18	103.9	0.9	184	yes	yes	105.0	2.2	180	yes	no?
22	102.8	0.4	260	yes	no	104.3	3.8	216	yes	yes

The three cats with symmetrical lesions (8, 20, 40) suffered a marked loss in ability to prevent overheating as measured by the respiratory responses to hot box tests one month after the operation (Table 7). In each of these the rectal temperature was raised to 106° without causing panting and without causing much increase in the respiratory rate. Later tests (Cat 40, 6 weeks; Cat 20, 10 weeks and Cat 8, 16 weeks) revealed little improvement in ability to resist overheating except that Cat 8 showed some increase in respiratory rate. However, 27 weeks after the operation Cat 8 was able to pant at 105.3°, thus showing a delayed and partial recovery of the capacity to regulate against heat.

The cats with asymmetrical lateral lesions showed an early loss in the ability to regulate against heat. In tests made one week after the operation Cats

Table 7 *Hot box tests on cats with lateral lesions in the rostral port of the hypothalamus Cats 8, 20 and 40 had symmetrical lesions, the other four had asymmetrical lesions Rectal temperature in degrees Fahrenheit*

Cat no	Preoperative tests					Tests one month postoperative				
	Temp at end of test	Rise in temp	Final respiratory rate	Pant ing	Sweat ing	Temp at end of test	Rise in temp	Final respiratory rate	Pant ing	Sweat ing
Sym										
8	104 5	3 2	176	yes	no	106 2	3 8	70	no	yes
20	104 4	2 3	230	yes	yes	106 0	7 7	26	no	no
40	103 3	1 3	246	yes	yes	106 0	2 8	36	no	no
Asym										
23	103 1	−0 2	206	yes	yes	103 9	2 6	220	yes	yes
39	102 7	0 4	252	yes	yes	105 9	3 7	140	yes	no
42	104 0	1 4	176	yes	yes	104 9	3 8	120	yes	yes
43	102 7	0 6	240	yes	yes	105 9	2 6	222	yes	no

39 and 43 reached temperatures above 106° without panting and with almost the same respiratory rates as at the beginning of the tests. The other two (23 and 42) did not pant at this temperature although the respiratory rate was high However, one month after the operation Cats 23 and 42 had so far recovered that the panting levels were less than 1° above the preoperative levels and Cats 39 and 43 panted at 105 9°. Cold box tests on the cats with symmetrical lesions showed greater than normal drops in temperature, though in the tests made one month after the operation the final temperatures reached were not abnormally low. The cats with asymmetrical lesions gave normal reactions in the cold box (Table 8).

It is evident from the data presented that symmetrical lesions in the lateral part of the rostral hypothalamus are much more effective than asymmetrical lesions in causing disturbances in temperature regulation but even symmetrical lesions do not cause great loss in the capacity to regulate against cold. But such symmetrical lateral lesions cause a profound and prolonged loss in the ability to regulate against heat

Laterally placed moderate sized lesions in the posterior part of the hypothalamus cause very great impairment in the ability to regulate against both heat and cold. The seven cats in this series had lesions at the level of the mammillary bodies extensively damaging the lateral hypothalamic area. In all but

Table 8   *Cold box tests on cats with lateral lesions in the rostral part of the hypothalamus Cats 8, 20 and 40 had symmetrical lesions. The other four had asymmetrical lesions. Rectal temperature in degrees Fahrenheit.*

	Tests one week postoperative					Tests one month postoperative				
Cat no.	Temp. at start	Temp. at end	Change	Shivering	Av. temp. of box	Temp. at start	Temp. at end	Change	Shivering	Av. temp of box
Sym										
8	102 6	97 4	−5 2	yes	40	103 0	100 6	−2 4	yes	39
20	99 5	97 1	−2 4	yes?	44	102 1	99 6	−2 5	yes?	42
40						102.7	99 7	−3 0	no	43
Asym										
23	101 7	100 4	−1 3	yes?	45	102 4	101 8	−0 6	yes?	39
39						102 1	101 8	−0 3	yes?	43
42	103 2	102 4	−0 8	yes?	44	101 8	102 4	+0 6	yes?	40
43						101 9	102 0	+0 1	yes	44
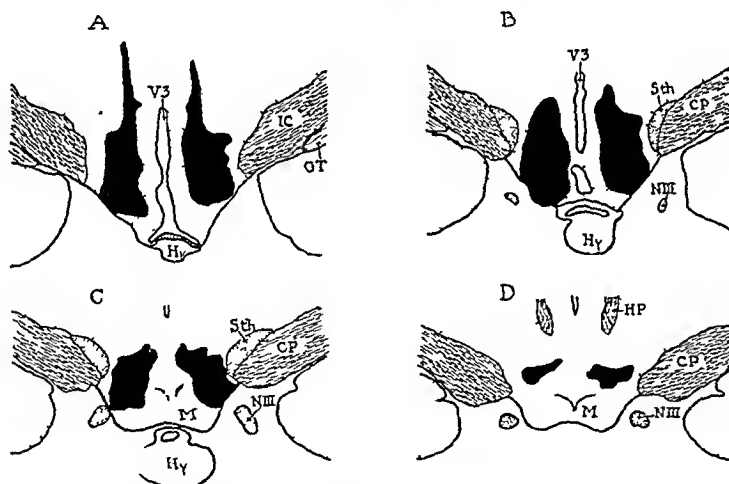
FIG 5. The lesions in Cat 47 indicated in solid black on four drawings from transverse sections through the brain at the level of and in front of the mammillary body.

one the lesions were fairly symmetrical bilaterally and in most instances they extended far enough dorsally to involve the fields of Forel and to interrupt any fibers which may run through the supramammillary decussation from the lateral hypothalamic area to the central gray matter. In Cat 47 (Fig. 5) the lesions were situated somewhat farther rostrally than in the others and extended from the level of the infundibulum to the level of the middle of the

mammillary bodies In Cat 110 the lesions were placed farther caudally (Fig. 6). In one cat (114) the lesions were quite asymmetrical one being in the lateral hypothalamic area on the left side, the other being in the midline leaving the right lateral hypothalamic area largely intact. These lesions would have interrupted in addition to the fibers descending on the left side into the mesencephalic tegmentum any fibers which may enter the central

gray matter through the supramammillary decussation. This cat showed marked disturbances in temperature regulation, a month after the operation but tests were not made after 2 or 3 months to determine to what extent recovery may have taken place.

On the morning after the operation these animals were lethargic. There was considerable extensor tonus especially in the hind legs and the cats could be molded into various bizarre postures similar to those assumed by the cataleptic cats pre-

FIG 6 The lesions in Cat 110 indicated in solid black on four drawings from transverse sections through the brain at the level of and behind the mammillary bodies

viously described (Ingram, Barris and Ranson, 1930). Usually within 4 days after the operation the catalepsy disappeared and the cats became alert and active and would eat spontaneously. There was no postoperative diarrhea such as occurred in many of the animals with lesions situated farther forward. On the morning after the operation they had, when removed from the incubator, rectal temperatures of 96° or less (Table 4). On the seventh day they all were able to maintain normal temperatures in the warm animal room except Cat 109.

In cold box tests all of these cats showed a marked decrease in ability to prevent a loss of body heat (Table 9). Five of the 7 showed in one or more of the tests drops of more than 7° as a result of 3 hours exposure to temperatures ranging from 34 to 44°. In 2 (Cats 100, 107) there was evidence of partial recovery of ability to keep warm with the lapse of time. But in the others practically no recovery occurred within the limits of time intervening between the first and last tests. Cat 47 showed a drop of 9.7°, 123 days after the operation; Cat 109 showed a drop of 7.8°, 68 days after the operation; and in Cat 110 the temperature dropped 3.5 to 96.6° in the test made 62 days after the operation. It will be obvious, therefore, that while most of the cats ran an

approximately normal temperature when kept in a warm room a week after operation, this cannot be regarded as evidence for a return of normal temperature regulation.

The postoperative hot box tests showed a marked loss in ability to regulate against overheating (Table 10). With rectal temperatures of 106° none of the cats panted and the respiratory rate remained slow. The information at hand does not necessarily show that the ability to pant was abolished by

Table 9. *Cold box tests in cats with lateral lesions in the caudal part of the hypothalamus. Rectal temperature in degrees Fahrenheit.*

	Preoperative tests					Postoperative tests					
Cat no.	Temp. at start	Temp. at end	Change	Shiv-ering	Av. temp. of box	Days after oper-ation	Temp. at start	Temp. at end	Change	Shiv-ering	Av. temp. of box
46	102.3	99.7	−2.6	yes	44	6	100.3	96.9	−3.4	yes	42
						41	100.4	97.0	−3.4	yes	40
47	101.3	100.0	−1.3	yes	44	18	102.6	93.6	−9.0	no?	44
						123	102.6	92.9	−9.7	no?	39
100	101.5	101.8	+0.3	yes	46	20	102.3	94.6	−7.7	yes	41
						55	102.6	98.4	−4.2	yes?	40
107	101.8	102.1	+0.3	yes	41	26	103.8	96.2	−7.6	no	37
						84	102.8	100.0	−2.8	no	35
109	101.9	101.7	−0.2	no	42	33	102.3	98.0	−4.3	no	37
						68	102.5	94.7	−7.8	no	36
110	101.1	100.3	−0.8	yes	36	32	101.0	96.7	−4.3	no	41
						62	100.1	96.6	−3.5	no	36
114	101.7	101.0	−0.7	no?	34	33	102.7	94.6	−8.1	no	34

these lesions. Certainly the threshold for panting was raised above 106°, but in those cats in which the rectal temperature was forced sufficiently high, panting occurred. In a test made on Cat 46, 49 days after the operation, the box temperature was raised to 114° and panting began when the rectal temperature reached 109.7°. This test was made long after the operation and it is possible or even probable that had a similar effort been made to force panting within a week or so after the operation the animal's temperature would have reached a fatal level before panting occurred.

Medially placed moderate sized lesions in the caudal part of the hypothalamus. caused little disturbance in temperature regulation. There were 3 cats with unilateral lesions destroying one mammillary body and one cat (103) with bilateral lesions destroying both mammillary bodies. The temperatures of all these cats during the postoperative days were normal except that one cat (105) with a unilateral lesion had a subnormal temperature on the first post-

operative morning. Hot and cold box tests made from 2 to 4 weeks after the operation were normal Earlier tests were not made in any of these cats except in one (Cat 25) with unilateral destruction of the mammillary body. On the second postoperative day this cat failed to pant in the hot box although

its respiration was 156 when its temperature reached 106°. In a later test this cat reacted normally in the hot box and the poor performance on the second day is to be explained by a transient impairment of function in regions outside the anatomical lesion.

The chief interest lies in Cat 103 with bilateral destruction of the medial part of the caudal hypothalamus (Fig. 7). The lesions destroyed all of both mammillary bodies except the rostral tip of the one on the right and every thing dorsal to the mammillary bodies as far as the floor of the
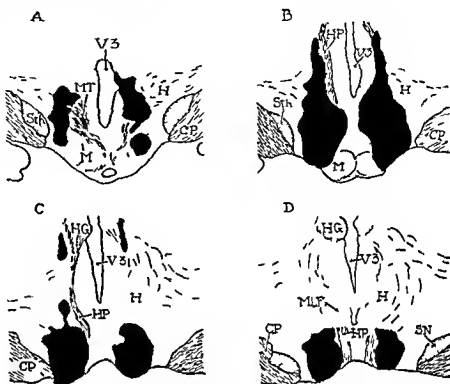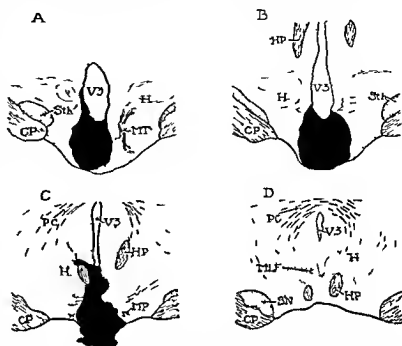

Fig 7 The lesions in Cat 103 indicated in solid black on four transverse sections through the brain at the level of and behind the mammillary bodies

Table 10 Hot box tests on cats with lateral lesions in the caudal part of the hypothalamus
Rectal temperature in degrees Fahrenheit

	Preoperative tests						Postoperative tests				
Cat no	Temp at end of test	Rise in temp	Final respiratory rate	Panting	Sweating	Days after operation	Temp at end of test	Rise in temp	Final respiratory rate	Panting	Sweating
46	105 1	2 1	208	yes	no	7 20	106 2 106 0	8 4 7 5	42 30	no no	no no
47	103 4	1 6	300	yes	no	7 42	106 0 106 0	3 2 3 0	26 28	no no	no no
100	102 0	−0 1	240	yes	yes	28	106 1	3 5	66	no	no
107	103 3	1 1	230	yes	no	26 42	106 3 106 3	6 2 3 4	30 40	no no	no no
109	103 4	1 1	180	yes	no	33 66	106 0 106 0	5 4 4 1	20 36	no no	no no
110	101 8	0 9	210	yes	yes	32 56	106 3 106 0	3 9 4 4	30 30	no no	no no
114	102 5	0 9	240	yes	yes	35	106 0	1 9	140	no	no

third ventricle. In this cat the lesion was in a position to interrupt fibers which descend from the hypothalamus through the central gray matter of the aqueduct; but the main pathway which runs lateral and dorsolateral to the mammillary body was intact on both sides. This cat showed no disturbance in temperature regulation so far as could be determined by the daily temperature records and by the hot and cold box tests made 30 days after the operation. It is quite possible that had these tests been made within a day or two after the operation transient abnormalities would have been detected.

DISCUSSION

It is difficult to make accurate observations on sweating and shivering. Sweating, which in the cat occurs only on the pads of the feet and is never profuse, is often obscured by evaporation. In the preoperative tests shown in Table 10 only 3 of the cats were observed to sweat and 4 were not. Hence no great importance can be attached to the fact that in the postoperative tests shown in the same table none of the cats were observed to sweat. It is not always easy to tell whether or not a cat is shivering. In the postoperative tests shown in Table 9, Cat 47 showed some twitching of the muscles which did not feel to the observer's hand like shivering and the observation was entered as a questionable negative. Shivering was not always detected in the preoperative tests. Two of the cats with lateral lesions in the caudal part of the hypothalamus did shiver during the postoperative tests. Hence it is not possible to say that these lesions abolished shivering although shivering occurred less often in the operated than in the unoperated cats.

The *delayed hypothermia* which developed after the lapse of from 7 to 10 days as a result of large anteriorly placed lesions (Table 1) in cats which had had normal or higher than normal rectal temperatures during the first postoperative days is difficult to explain. It differs from the hypothermia caused by more posteriorly placed lesions in that the latter appears promptly after the operation and decreases with the lapse of time. The explanation for this delayed hypothermia may perhaps in some instances lie in a toxic depresson of the rest of the hypothalamus due to the diffusion of substances formed in the lesions.

It does not seem probable that the explanation could lie in the malnutrition resulting from the refusal of the animals to eat. To test this possibility, normal cats have been deprived of food for many days and it was found that their capacity to regulate against cold was not impaired until they had lost 30 per cent or more of their body weight (Clark, 1938). Few of the cats with preoptic lesions were as emaciated as this since they all received 100 cc. of milk daily by stomach tube. One of the cats (no. 97) had a rectal temperature of 97°, 60 days after the operation although at this time it was eating well and was in good physical condition. Incidentally the existence of hypothermia in this animal 60 days after the operation also speaks against the theory of a toxic depression of the hypothalamus in so far as the debris from the preoptic lesions would have been absorbed by this time. It must be admitted that no satisfactory explanation for this delayed hypothermia is available at present.

Increased peristaltic activity of the intestines, as evidenced by the passage of large amounts of soft stools during the night following the operation, diarrhea, and violent defecatory movements induced by the insertion of the thermometer was seen in a considerable number of the animals especially in those with large anteriorly placed lesions. If this were due entirely to elimination of sympathetic inhibitory tonus emanating from the hypothalamus it should have appeared more frequently in the animals with lesions in the middle and posterior parts of the hypothalamus. This would seem to indicate that stimulation of the gut is involved but no information is furnished about the source of such stimulation.

Diarrhea frequently develops in cats with hypothalamic lesions when because of their refusal to eat spontaneously they are subjected to tube feeding with 100 cc. of milk daily. Normal cats when deprived of all food except 100 cc. of milk daily do not develop a diarrhea whether they drink the milk or have it given them by tube. The milk diet may cause them to have soft stools but not a diarrhea.

Chilling. Little if any information concerning the localization of heat regulating centers can be obtained from the experiments in which very large lesions were made. The smaller lesions are much more instructive. Subnormal temperatures were observed on the first postoperative morning in none of the cats with medially placed lesions and in only one of the cats with laterally placed lesions in the anterior hypothalamus (Cat 20, Table 4). The cats with moderate sized laterally placed lesions in the posterior part of the hypothalamus showed a marked hypothermia the first morning after the operation, but there was a rather rapid recovery so that on the 10th day only two of them had temperatures below 101° (Table 4). It would appear that cats recover from the hypothermia caused by hypothalamic lesions more rapidly than do monkeys; but even monkeys regain the ability to maintain normal body temperatures under ordinary room conditions in a few weeks (Ranson, Fisher and Ingram, 1937).

But rectal temperatures of animals kept under ordinary room conditions do not furnish a satisfactory measure for their ability to regulate against cold. For this purpose cold box tests are required. Such tests made during the first postoperative week gave normal results in cats with medially placed anterior lesions (Table 5) and abnormally large drops in cats with symmetrical lateral lesions in the anterior part of the hypothalamus. Even after one month these cats with symmetrical anterolateral lesions still showed falls in temperature which were considerably greater than normal (Table 8). By far the greatest chilling, however, was seen in the cold box tests on cats with laterally placed lesions in the posterior part of the hypothalamus (Table 9).

It is particularly significant that these cats with posterolateral lesions showed little tendency to recover the ability to prevent chilling in the cold box, the temperature of Cat 47 falling 9.7° in three hours in a box at 39° in a test made 123 days after the operation. In 2 of the cats listed in Table 9 there was evidence of some recovery. But in one of these (Cat 100) the lesions were somewhat asymmetrical and in the other cat (107) the lesions were much

smaller than in the other cats of this group. The recovery of ability to resist chilling in the cold box shown by these cats was evidently due in large part to the incompleteness of the lesions.

Considerable difference of opinion exists with regard to the extent to which subsidiary centers are capable of contributing to temperature regulation in the absence of the hypothalamus. Keller (1933) and Thauer and Peters (1937) found recovery of ability to maintain a normal body temperature under ordinary environmental conditions in chronic midbrain animals, though a normal regulation against extremes of external temperature was never regained. The perfect protection against cold offered by the fur of the rabbit may in part account for Thauer's (1935) finding that some time after the transection of the cervical cord that animal was able to preserve body temperature in a fairly normal manner, for his results are not in accord with those of Sherrington on the dog (1924) or Clark (1939) on the cat.

Overheating. Hyperthermia was present in many of the cats on the morning after the operation. In the group with large anterior lesions 9 out of 20 cats had temperatures above 104.2°. Four out of 8 cats with medially placed moderate sized lesions in the anterior hypothalamus and 6 out of 8 cats with laterally placed moderate sized lesions in the anterior hypothalamus also had temperatures above 104.2 on this first morning. These high temperatures can scarcely be attributed to nonspecific results of the operation, because in a group of cats in which similar lesions were made in the thalamus by the same method the temperatures on the first postoperative morning were 102.1, 102.3, 103.6, 103.7 and 104.1° respectively. In these as in all the cats with hypothalamic lesions the rectal temperatures were taken on the first morning at the time the cats were removed from the incubator in which they had been kept over night so that the factor of environmental temperature was essentially the same in all cases. Under these conditions normal cats regulated their temperatures perfectly and many of the cats with hypothalamic lesions showed subnormal temperatures. The hyperthermia seen in some of the cats cannot, therefore, be attributed to the high temperature of the incubator.

The location of the lesions in the animals with high temperatures on the first postoperative morning have varied a good deal. Large lesions in front of the anterior commissure (Cats 53 and 54), at the level of the anterior commissure (Cats 52 and 92) or in the suprachiasmatic hypothalamus (Cats 27 and 81) and smaller lesions either medially placed at the level of the infundibulum (Cats 11 and 18) or in the anterior part of the lateral hypothalamus (Cats 8 and 42) have frequently caused hyperthermia. It would seem most reasonable to attribute the rise in temperature to an irritation of the mechanism for heat conservation and heat production since in none of these animals was most of the hypothalamus destroyed and the most distant lesions were within 2 mm. of it. Many of these cats showed impaired capacity to regulate against heat as measured by the hot box tests, and this probably was a factor in permitting the high temperatures to develop. But this factor is not in itself sufficient to cause hyperthermia. In Cats 8, 20 and 40 the impairment of capacity to prevent overheating persisted for at least a month but

hyperthermia lasted for only a few days in 8 and 40 and did not appear in 20.

The capacity to regulate against overheating as measured by the hot box test was very seriously disturbed in all but one of the cats with large anteriorly placed lesions which were subjected to this test. It is significant that in all but this one the region dorsal to the optic chiasma was extensively damaged. A month or more after the operation these cats failed to pant or to show much of an increase in respiratory rate when their temperatures were raised to 106° or higher (Table 3). The exception was Cat 53 in which the lesions were situated in front of the anterior commissure and a large part of the preoptic region remained intact. Cats from which the frontal lobes had been removed for another purpose (Magoun and Ranson, 1938) also showed normal reactions in the hot box.

Cats with smaller medially placed lesions in the anterior part of the hypothalamus showed after one month only a moderate increase in the panting level and in respiratory rate (Table 6). But cats with moderate sized symmetrical lesions in the lateral part of the anterior hyopthalamus failed to pant and showed little increase in respiratory rate when their rectal temperatures had been raised to 106° one month after the operation (Table 7, Fig. 4). All of the cats with laterally placed lesions in the posterior part of the hypothalamus also showed greatly impaired capacity to regulate against heat (Table 10). This is in part a confirmation and in part an extension of the observations of Teague and Ranson (1936).

It has been found that a region specifically responsive to heat exists above the chiasma and in the neighborhood of and below the anterior commissure. Heating of this region with a high frequency current led in over a pair of enameled nichrome wires causes panting and great increase in respiratory rate (Magoun, Harrison, Brobeck and Ranson, 1938). The results of the present investigation on the effects of lesions agree very well with those obtained by heating the brain if it is assumed that the "center" in the suprachiasmatic and preoptic region, which is sensitive to heat, sends fibers backward by way of the medial forebrain bundle in the lateral hypothalamus, which are interrupted by such lesions as those illustrated in Fig. 4, 5 and 6. It must be emphasized that although the suprachiasmatic and preoptic regions are sensitive to heat they do not contain the coordinating motor mechanism for panting, because decorticate polypneic panting can occur after these regions have been removed (Lilienthal and Otenasek, 1937). This motor mechanism occupies a more caudal position perhaps in the mesencephalon (Keller, 1933).

From the information at hand the most probable explanation of the transient hyperthermias seen in cats, monkeys (Ranson, Fisher and Ingram, 1937) and man after lesions in the anterior hypothalamus and the suprachiasmatic region is that these lesions cause an irritation of the centers for heat formation and heat conservation in the hypothalamus and that the resultant rise in temperature fails to activate the heat loss mechanism since the corresponding center has been destroyed or at least cut off from lower lying centers. Bilateral destruction of the caudal part of the lateral hypothalamus by large lesions

like those shown in Fig. 5 largely destroys the centers for heat formation and conservation and interrupts the descending paths from these centers as well as those from the center in the suprachiasmatic and preoptic region which is essential for regulation against heat. Such lesions cause a loss in the capacity to regulate against both heat and cold.

SUMMARY

Large lesions in the hypothalamus in cats cause marked disturbances in temperature regulation and frequently result in the death of the animal. The farther back the hypothalamic lesions are placed the greater is the impairment of the ability to keep the body temperature up to the normal level and the more rapidly does death occur.

Large lesions in the region dorsal to the optic chiasma and ventral to the anterior commissure may cause serious impairment in the ability to prevent overheating without much disturbance in the ability to prevent chilling.

Bilateral lesions in the anterior part of the lateral hypothalamus cause a moderate impairment in the ability to prevent chilling and marked impairment in the ability to prevent overheating.

Bilateral lesions in the caudal part of the lateral hypothalamus have caused marked impairment in the ability to prevent both overheating and chilling.

Moderate sized medially placed lesions which do not invade the lateral hypothalamus have not caused much disturbance in temperature regulation irrespective of whether they were situated at the level of the infundibulum or at the level of the mammillary body.

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SIMILARITY OF EFFECTS OF BARBITURATE
ANESTHESIA AND SPINAL TRANSECTION

H K BEECHER, F K McDONOUGH, AND A FORBES

*From the Surgical Laboratory of the Massachusetts General Hospital and the
Department of Physiology of the Harvard Medical School, Boston*

DURING some recent studies of the influence of anesthetic agents upon the
electrical activity in the cerebral cortex of cats, we used the flexion reflex,
initiated by a series of induction shocks applied centrally to the sciatic nerve,
as a rough measure of anesthetic depth Under ether (Fig 1, A, D), as in the
decerebrate cat, a record of lower leg flexion shows a cumulative contractile
effect With a number of agents, notably the barbiturates, the character of
the contractions was greatly altered (Fig 1, B, C) In this case, scarcely any
cumulative contraction occurred, rather, each stimulus was followed by iso-
lated and approximately equal twitches, rising from and returning nearly to
the base line The similarity of these observations to those found by Forbes,
Cobb and Cattell (1923) before and after low transection of the spinal cord,
was obvious The present study was planned to analyze this observation and
to determine in what respects the effect of a barbiturate on the flexion reflex
was similar to that of low spinal transection

APPARATUS AND METHOD

Animals Cats were used in all experiments, with artificial heating to maintain body
temperature For recording the flexion reflex, either the tibialis anticus or the hamstring
muscles were used Stimuli were applied to the central end of the severed sciatic nerve in
the case of the hamstrings, and the popliteal nerve for tibialis anticus The stimulating
electrodes were silver wires mounted in a rubber tube The stimulating apparatus consisted
of a Harvard inductorium activated by a 1 5 volt dry cell and operated either by a mercury
contact key manipulated by hand or by a rotary interrupter
  The time of stimulation was shown on the record by a string galvanometer signal
device The stimuli were single make or break induction shocks spaced from one to several
seconds apart and followed by a rapid series of shocks (by hand) The strength of stimulus
employed was that which would evoke approximately a maximal flexion reflex When a
rotary interrupter was used in the primary the frequency of stimulation, counting both
makes and breaks, was from 7 to 10 per see For the *isotonic* preparation the writing lever
was attached by cord to the ankle Its excursions were restrained by an elastic band The
flexion response was recorded on a smoked drum In order to obtain more faithful records
of muscular contraction than is possible with the isotonic preparation, we used an *isometric*
lever in several experiments In the isometric preparation the hip joint of the cat was rela-
tively immobilized by section of the femoral nerve, the psoas muscles and the glutei The
proximal and distal ends of the femur were pierced by drills The leg was held fixed by in-
serting the drills into rigid clamps Tibialis anticus was freed distally and a hitch of tendon
taken about a steel hook This was later applied to the torsion myograph described by
Forbes, Davis and Lambert (1930) The sciatic nerve was dissected, and the branch to the
hamstrings severed The popliteal and peroneal divisions of the sciatic nerve were freed,
and popliteal severed near the knee for stimulation When the hamstring muscles were
employed, essentially the same preparation was used, the nerve to this group being spared.
  Muscle action currents Freshly chlorided silver electrodes were inserted into tibialis
anticus through small skin slits about one centimeter apart, at the mid portion of the

muscle. Here also, the proximal lead was attached to the ground. The muscle electrodes were placed in circuit with the direct coupled amplifier described by Forbes and Grass (1937) connected with a string galvanometer. The potential changes between the leads were recorded on the same film as the myograph. A timer with 10 msec. units recorded on one margin of the film.

Nerve action currents. In an attempt to get further confirmatory evidence of the differences under the two agents, we recorded in several animals the action currents in the peroneal nerve, which appeared in response to stimulation of the popliteal. For this purpose the peroneal division of the sciatic nerve was severed near the knee and crushed one centimeter proximally to render the action currents monophasic. The nerve was placed in a moist chamber containing freshly chlorided electrodes. The proximal electrode was connected with the ground. The distal electrode (grid lead) supported the nerve between the cut end and the crushed area.

Anesthetics. Ether was administered by tracheal cannula. "Evipal" (1-methyl-5Δ′ cyclohexenyl-5-methyl-barbituric acid) was administered either intravenously or intraperitoneally in doses of 10 to 40 mg. per kilo. The smaller doses were repeated at 10 to 30 minute intervals. This particular barbiturate was chosen because it is swiftly detoxified and recovery of the animal from its influence is relatively rapid. This made it possible to carry out a series of studies upon the same animal, alternating ether and "evipal" anesthesia. About 40 minutes were required for elimination of the ether after its administration was interrupted. If only very light "evipal" anesthesia was induced, the drug was rather completely destroyed in about this same length of time. While three alternations of agents were made in some cases, giving two series of observations under ether and two under "evipal" for comparison in the same animal, we found that the best data were obtained from the first ether and the first barbiturate observations.

RESULTS

Comparison of ether with barbiturates

Isotonic method. Typical examples of these effects are shown in Fig. 1; the four records were obtained in sequence in the same animal (stimuli induction shocks, by hand). In A, B, and C isolated shocks preceded the rapid series. In D the response to a rapid series alone is shown. Figure 1A was recorded at 2:56 p.m. under light ether anesthesia, the administration having previously been curtailed. Figure 1B was obtained at 3:20, 4 min. after a 30 mg. per
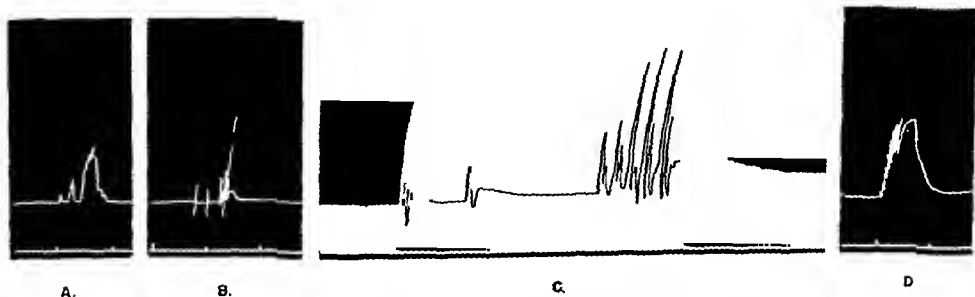
FIG. 1. Isotonic muscular contraction with flexion of the lower leg of the cat following induction shocks. Lower line, time in 5 sec. units. In A, B, and C 2 isolated shocks were followed by a rapid series; in D a rapid series only. A, under light ether anesthesia; B, later under light "evipal" anesthesia; C, the same as B with anesthesia a little lighter, fast drum; D shows resumption of typical ether response after return to that agent. A and D show the cumulative contraction under ether anesthesia. B and C show the discrete nature of the response found under a barbiturate ("evipal").

kilo dose of "evipal" had been administered. Figure 1C was observed at 3:26 and recorded on a fast drum, under somewhat lighter "evipal" anesthesia than was the case in B, as is indicated by the greater flexion response. Figure 1D was obtained at 5:14, 34 min. after ether anesthesia had been resumed (and 1 hr. and 58 min. since the barbiturate had been administered). This figure shows clearly the cumulative contraction under ether, on the one hand, and the isolated nature of the twitches under "evipal" on the other.

In a study of cortical action potentials under a considerable number of anesthetic agents in which the flexion reflex was used incidentally as a measure of depth of anesthesia, we have found the flexor response to be similar to that under ether [9] in the following*: nitrous oxide [4], ethylene [3], cyclopropane [4], chloroform [7], ethyl chloride [3], alcohol [4], amylene hydrate [3], trichlorethylene [4], urethane [4]. The following have yielded records like "evipal" [6], sodium barbital [4], "nembutal" [6], and chlorolosane [4]. The three barbiturates studied are examples of the so-called slow, moderate and fast acting barbiturates. It is probable that anesthesia by all members of this chemical family would result in the same alterations of the flexor response. The remainder have not fallen as clearly into the two groups. Whether the flexor response of these agents resembles the ether group or the barbiturate group seemingly depends upon the depth of anesthesia. In this intermediate group we have found tribromethanol [4], divinyl ether [4], and paraldehyde [4].

Isometric method. These are shown in Fig. 2, 3 and 4. Each figure was obtained from a different animal; the successive records of each figure were made chronologically from the same animal. In each case when one agent followed the other, ample time was allowed for the elimination of the ether or the destruction of the barbiturate. Figure 2 shows (preparation under ether anesthesia) the isometric response of tibialis anticus to induction shocks (by hand) at the rate of about 12 per sec., counting both makes and breaks, and of a strength to give approximately a maximal response on the break and a submaximal on the make. A brief protocol for Fig. 2 follows.

11 00 A M.	Ether anesthesia started
1 00 P M	Record A obtained, under light ether anesthesia
1 15 P M	Ether anesthesia discontinued
1 20 P M	"Evipal" injected intravenously, 30 mg per kilo body weight
2 37 P M	"Evipal" injected intravenously, 10 mg per kilo body weight
2 45 P M	Record B obtained under deep "evipal" anesthesia
3 10 P M	Record C obtained under light "evipal" anesthesia
3 12 P M	Ether anesthesia resumed
3 43 P M	Record D obtained under moderately light ether anesthesia

It will be seen here, as in the case of the isotonic records, that this experiment also reveals a transition from sustained or cumulative contraction under ether, to isolated twitches under "evipal." It is noteworthy that with "evipal," even under deep anesthesia, the contractile response to each stimulus in the series appears as a larger excursion from the existing level than is the case during ether anesthesia.

Figure 3 shows (after preparation under "evipal") the typical barbiturate

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* The number of experiments performed is indicated in brackets after each agent.

Fig. 2. Isometric contraction of tibialis anticus muscle (cat) in response to a series of induction shocks (by hand); preparation under ether anesthesia. Interval of stimulation is indicated by the solid line below the record, and the time unit is shown immediately above each record. Read from left to right. In all cases the upper tracing of each record represents the response of the galvanometer string to action currents in the muscle. An upward excursion of the string represents a negative potential of the proximal lead, in all cases unless otherwise specified. The lower tracing is that of the myogram: a rise in this signifies an increased muscle tension. A. 1 p.m. light ether anesthesia. B. 2:45 p.m. deep "evipal" anesthesia. C. 3:10 p.m. light "evipal" anesthesia. D. 3:43 p.m. moderately light ether anesthesia. The characteristic cumulative ether response is shown in A. The isolated, muscular twitches characteristic of a barbiturate are shown in B and C, while D shows, with the resumption of ether anesthesia, the return of the ether response.

isometric response of tibialis anticus to induction shocks (by hand) at the rate of about 5 per sec., counting only breaks. The breaks were approximately maximal in effect. A brief protocol for Fig. 3 follows.

1:45 P.M.	"Evipal" administered intravenously, 30 mg. per kilo body weight
2:20 P.M.	"Evipal" administered intravenously, 20 mg. per kilo body weight
3:25 P.M.	"Evipal" administered intravenously, 10 mg. per kilo body weight
3:55 P.M.	"Evipal" administered intravenously, 10 mg. per kilo body weight
4:25 P.M.	Record A obtained under light "evipal" anesthesia
4:27 P.M.	Ether anesthesia started
5:42 P.M.	Record B obtained under light ether anesthesia

Here again, these records show the typical isolated twitches of the flexion response under "evipal" in contrast to the cumulative sweep found under ether. This figure shows even more strikingly than the preceding one (Fig. 2)
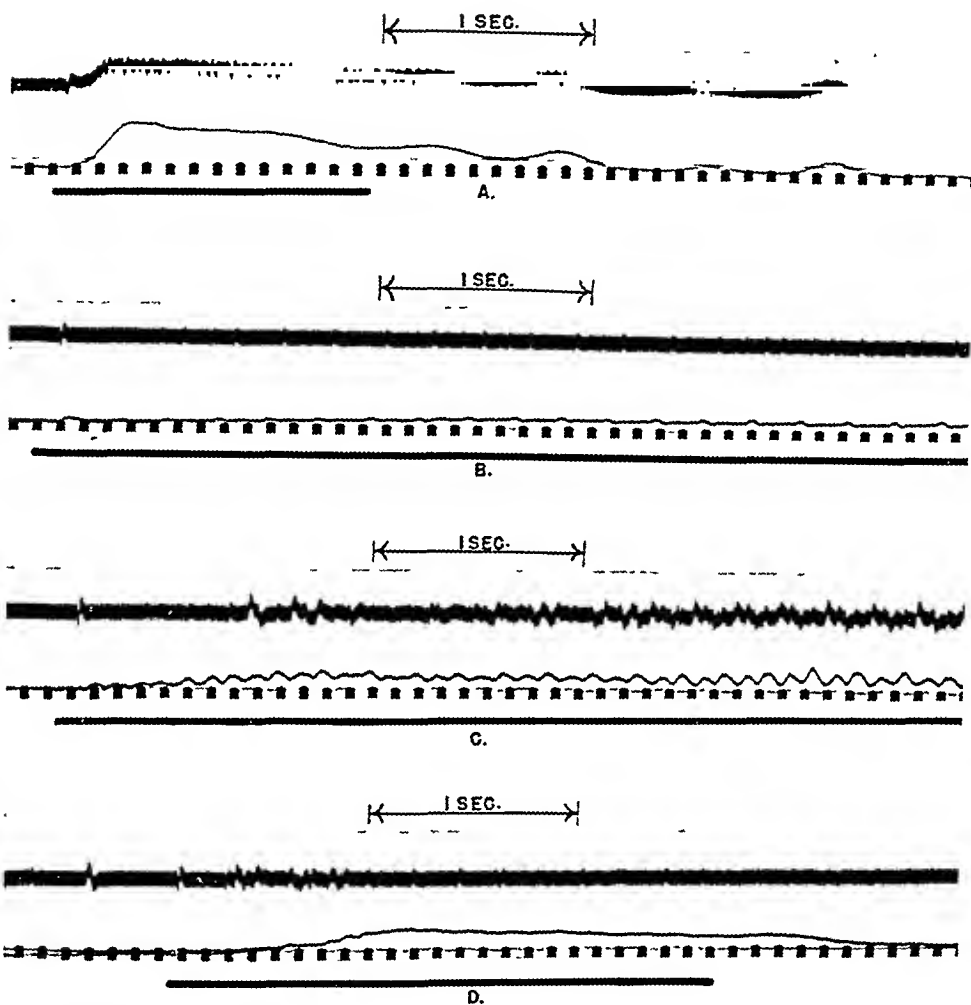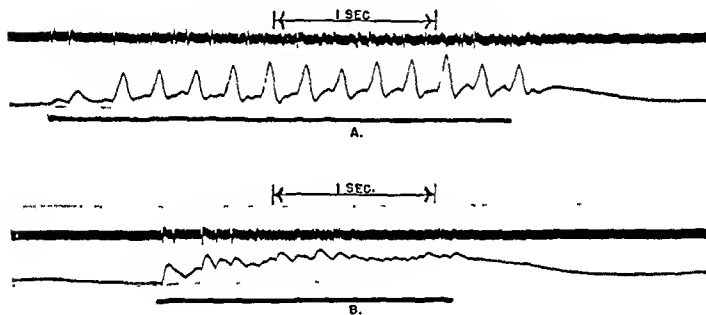

FIG. 3. Isometric contraction of tibialis anticus muscle in response to a series of induction shocks (by hand). Details are the same as for Fig. 2. Preparation under "evipal" anesthesia. A. 4:25 p.m. under light "evipal" anesthesia. B. 5:42 p.m. under light ether anesthesia.

the greater contractile effect of the individual twitches under "evipal" than under ether.

Figure 4 shows the response of the hamstring muscles (following an ether preparation) to induction shocks set up regularly by means of the rotary interrupter. The frequency of stimulation counting both makes and breaks was 10 per sec., in A and 8 in B. These shocks gave approximately a maximal muscle response. In this case, unlike the preceding, a downward excursion of the galvanometer string signifies negativity of the proximal lead. Ether anesthesia was started at 9:45 a.m. and maintained continuously until Record A was obtained at 1:23 p.m. The typical cumulative response of the flexion reflex under ether is shown. Ether administration was terminated at 1:20,

and 40 mg. per kilo. body weight of "evipal" were given intravenously at 1:27. Record B of Fig. 4, obtained at 2:11 p.m., shows the characteristic muscle response under a barbiturate.

The action current records of the *muscle* supplement the data by showing more clearly the more discrete responses under the barbiturate than under ether, in which often an irregular sequence of small excursions appears not correlated with the stimuli. In recording the *nerve* action currents we hoped to be able to demonstrate, in response to a rapid series of stimuli, a quick falling
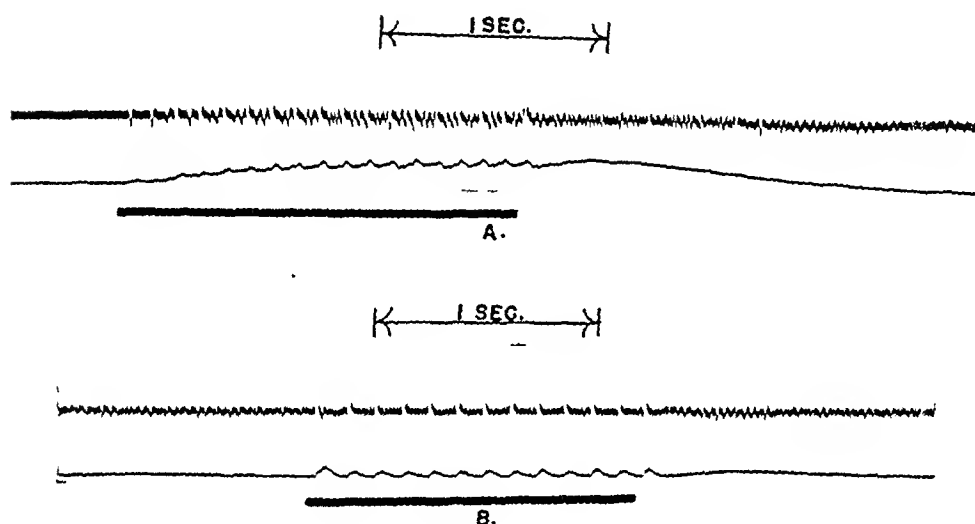
FIG. 4. Isometric contraction of the hamstring muscles in response to a series of induction shocks. Ether preparation. Details are the same as for Fig. 2 with the exception that here a downward excursion of the galvanometer string signifies negativity of the proximal grid lead. And also in this case, the induction shocks were spaced by a "rotary" interrupter. A. 1·23 p.m. ether anesthesia. B. 2:11 p.m. "evipal" anesthesia.

off in the size of the electric response, under ether anesthesia, and under the barbiturate a sustained amplitude of response, these effects being comparable to those found before and after spinal transection by Forbes and his associates (1923, 1929). In this attempt it was found impossible to compare responses under precisely comparable levels of anesthesia. In view of this the attempt was abandoned.

The figures demonstrate repeatedly both isotonically and isometrically the sustained cumulative contraction in the flexor response to stimuli under ether as contrasted with the relatively isolated twitches under the barbiturate "evipal." The similarity of the change in picture encountered in these experiments to that obtained by Forbes, Cobb and Cattell (1923) upon spinal transection at the level of the last rib is thus demonstrated. The outstanding

difference between the mechanical responses in the case of the two anesthetic agents appears to be the same as the difference arising from low transection of the spinal cord namely, after transection, or under barbiturate anesthesia, the after-discharge is greatly reduced and the response to stimuli discrete, whereas, before transection, or under ether anesthesia, the relatively great after-discharge leads to a cumulative effect of a rapid series of stimuli and the curve of the response shows a sustained rise These findings are in harmony with the conclusions of Bremer from his studies on a frog's spinal reflex (1934), and on the response of the cat's cerebral cortex to acoustic stimuli (1937)

The view is widely held (Forbes, 1922, Lorente de No, 1938) that after-discharge is due to internuncial neurones which, through long-circuiting of sensory impulses, provide for continued stimulation of the ventral horn cells after the original excitation has ceased In the decerebrate animal and in the animal under ether the long-circuiting apparently is possible, but not so when the spinal cord is physically transected or the animal under the barbiturate anesthesia One might infer, then, that the long circuiting of sensory impulses is more greatly reduced under the barbiturate than under ether

While attention has been drawn to the similarity of effects, it is not our intention to imply that the action of the barbiturates is similar to the effects of spinal transection in all respects Doubtless many differences could be shown In the features noted the changes are surprisingly alike and, we hope, offer data that may be of use in adding to the meager store of information concerning the site of action of various anesthetic agents *

SUMMARY

The striking similarity of the effects of low spinal transection and barbiturate anesthesia upon the flexion reflex of the leg is reported Isotonic and isometric muscle studies with muscle action currents have been used to demonstrate that under ether anesthesia a relatively sustained, cumulative reflex contraction of the leg flexors is regularly found upon repeated central stimulation of the sciatic nerve or its popliteal division, whereas, under barbiturate anesthesia ("evipal") *larger* isolated noncumulative twitches of the muscle occur in response to stimulation The difference appears to be a matter of after discharge, great in the case of ether, but little under barbiturate anes thesia From this it is inferred that the "long-circuiting" of sensory impulses is much more seriously curtailed under barbiturate anesthesia than under ether The behavior of the flexion reflex of the leg under 15 other anesthetic agents is reported

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* The observations recorded here perhaps offer an explanation of the finding that the hind legs of dogs going under barbiturate anesthesia or recovering from it appear to be considerably more ataxic than the forelegs On the other hand this phenomenon may be merely a demonstration of innate poorer coordination in the case of the hind legs

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A STYLE BOOK CONTAINING SUGGESTIONS
FOR THE PREPARATION OF
MANUSCRIPTS

I. GENERAL DETAIL

Style and brevity

A WELL-KNOWN journal devoted to physiology has recently offered its contributors the following friendly advice: "Authors are asked to aim at writing papers as briefly as possible. Brevity, as a rule, accelerates publication, reduces editorial work and the cost of printing, and, lastly, lightens the burden of the reader. Errors from excessive brevity are more easily repaired than those from prolixity. It is important to remember that a reader's impression of a paper is often influenced by its literary style, and in his own interests the author should take pains about his style in order to convey his meaning to his readers. Care should be used in the choice of appropriate words and their place in the sentence, as well as in the sequence and linkage of sentences. A. P. Herbert's *What a Word!* and H. W. Fowlder's *Modern English Usage* may here be found helpful." To all this the editors of the *Journal of Neurophysiology* heartily subscribe.

In planning a paper material should be arranged, preferably with suitable headings, in the following order: (i) Introduction, stating the objects of the research and a brief history of the subject; (ii) Methods employed (in small print); (iii) Results, with illustrative protocols, diagrams or tables; (iv) Concise discussion; (v) Summary (preferably enumerated); (vi) Acknowledgments (small print); (vii) References; (viii) Legends for figures; (ix) Footnotes, when not typed in the body of the text; and finally (x) Illustrations with lettering complete.

Copy

The manuscript should be copied uniformly with *triple spacing* for parts to be printed in large type, with a margin of at least 1.25 inches (3 cm.) all around. The parts to be printed in small type should be typed on separate sheets in *double spacing*. The original typewritten copy should be packed flat for the mail or express. Corrections of the typescript should be typewritten or written legibly *in ink*. Manuscripts should be consistent in style; *i.e.*, a word should not be abbreviated on one page and written out on the next, nor temperature given in Fahrenheit in one place and Centigrade in another. Coöperation in making manuscripts as consistent as possible before submission for publication will greatly reduce editorial labor and the cost of alterations in the proof.

Preliminary matter

Title. The complete title of the paper in capitals together with the author's name(s) should appear on a *separate* first page; below this the institution

from which the paper emanates, the latter being underlined for italics. The usual arrangement is as follows:

THE POTASSIUM AND WATER CONTENTS OF CAT NERVES AS AFFECTED BY STIMULATION

WALLACE O. FENN

*Department of Physiology, School of Medicine and Dentistry,
The University of Rochester, Rochester, N. Y.*

Running title. The second page of the manuscript should give an abbreviated form of the title, not exceeding 40 letters in length, to be used as a running headline, together with the exact address to which the proofs are to be sent. Any other special directions to the printer can be included on this page.

Headings

Major headings, such as INTRODUCTION, METHODS, DISCUSSION, SUMMARY and REFERENCES are printed in capitals. Minor headings, whether center or side, and descriptive matter in table headings, are printed in italics and therefore should be underlined *once*.

Text

The text (including center headings) should be typed on consecutive pages until; (i) an experiment, (ii) a protocol, (iii) a table, (iv) a description of technical procedures, (v) a quotation of over five lines, (vi) a formula or equation, or (vii) description of technical procedure. *Begin every experiment, protocol, table, description of technical procedure, quotation of over five lines, formula, equation, or technical procedure, on a new page, regardless of where the preceding text ends on the page.* After the experiment or table, etc., has been copied on one or more sheets, proceed with the text on a new sheet until the next experiment or table, etc., is reached, when the same procedure is repeated.

This method brings the material of the entire manuscript in sequence, but permits, without mutilation of the manuscript, the separation in the printer's office of tables, and all other small type, which are set up separately by hand or on separate monotype machines. *Authors are requested to mark in the margin those portions of the paper that should be printed in small type.* The sheets should be numbered consecutively throughout, with the place of insertion of each text-figure indicated on the copy in ink. In the text, tables and figures should always be referred to specifically by number, *i.e.*, Fig. 2, not "the following figure." It is obviously not always possible to insert a table or figure in the precise place requested when the paper is arranged in page form.

Spelling

All preferences of spelling and literary usage will be respected unless the manuscript is inconsistent; minor deviations will be corrected in the editorial office. Major editorial changes will ordinarily be submitted to the author.

Footnotes

Footnotes should be typed in single space, numbered consecutively throughout the text and preferably inserted immediately after the line of text which requires the footnote, no matter where it occurs on the page.

References

Verification of references. The *Journal* office takes care of reading the page proof, but can not verify references; responsibility for this must rest entirely with the author. Since references are useless unless correct, authors are urged to verify against the source every reference after it has been transcribed so that it may be complete and correct in each detail—spelling, especially of proper names and foreign titles, journals, date, volume number, page numbers, punctuation and, above all, accents.

Form. Three systems may be adopted for the arrangement of references: (i) The references are arranged and numbered in the order of their appearance in the text, (ii) or arranged alphabetically according to the name of the author, the text reference being the name of the author with the year of publication referred to or (iii) the alphabetically arranged list numbered consecutively. Manuscripts will be returned if references are not in correct form and complete. Text references are indicated by an exponential number or by date.

Form of citation. The form of citation for *journals* is indicated by the following example:

> (i) SHERRINGTON, C S Decerebrate rigidity, and reflex coordination of movements *J Physiol* , 1898, 22 319–332

Citations of *books* follow the same procedure except that for single volumes the total pagination should be indicated so that the extent of the book is obvious, for example:

> (ii) MAGNUS, R *Korperstellung* Berlin, J Springer, 1924, xiii, 740 pp

When a book is two or more volumes, total pagination is unnecessary as in the following:

> (iii) RAMÓN Y CAJAL, S *Histologie du système nerveux de l'homme et les vertébrés* Paris, Maloine, 1909–1911, 2 vols

Handbooks and special serials can be treated in the manner of a journal, *e g.*,

> (iv) FOERSTER, O Symptomatologie der Erkrankungen des Ruckenmarks und seiner Wurzeln *Bumke u Foersters Handb Neurol* , 1936, 6 1–448
> (v) BROUWER, B Certain aspects of the anatomical basis of the phylogeny of encephalization *Res Publ Ass nerv ment Dis* , 1934, 13 3–25

In all citations authors' names should be in capitals, followed by the title of the paper, journal (abbreviated, see below) underlined once, year, volume (arabic numerals) underlined once, initial and final pages (see example i). In the case of books the authors' names should likewise be in capitals, followed by the title underlined once, place of publication, publisher, date and

total number of pages or volumes (see examples ii and iii). Reference to a specific page should be included when the citation is mentioned in the text (thus, Parker, 1918, p. 216).

The journal abbreviations should be in accordance with the system used in *A word list of scientific periodicals published in the years 1900–1933*, 2nd ed., (London and New York, Oxford University Press, 1934.) A list of the abbreviated titles of journals frequently cited in neurophysiological work is given on pp. 93–99.

Illustrations

All illustrations, especially photographs, should be sharp, of clear and submitted ready for reproduction, *i.e.*, with *professional lettering*, mounting and arrangements of figures completed. *Each illustration must have printed on the back author's name, address and the figure number.* If the original of a figure is submitted, it is hoped that the author will also include a photograph for editorial use. This will mimimize the danger of loss or injury to an original (*e.g.*, kymograph record).

II. LIST OF USUAL ABBREVIATIONS*

Absolute	abs.	Henry	H.
Absolute degrees	°K.	Horse power	H.P.
Alternating current	A.C.	Hour	hr.
Ampere	A.		(but m.p.h.)
Ångstrom unit (10^{-8} cm.)	Å.	Hydrogen ion concentration	pH
Arterial pressure	A.P.	(negative logarithm of)	
Atmosphere	atm.	Kilogram	kg.
Atomic weight	at. wt.	Kilogram meter	kg.m.
Blood pressure	B.P.	Kiloliter	kl.
Boiling point	b.p.	Kilometer	km.
British Thermal Unit	B.TH.U.	Kilovolt	kV.
Calculated (in table headings)	calc.	Kilovolt ampere	kVA.
Calorie (large)	kcal.	Kilowatt	kW.
Calorie (small)	cal.	Liter	l.
Candle	c.	Maximum	max.
Candle power	c.P.	Megacycle per second	Mc./sec.
Centigrade	C.	Megohm	MΩ. or MO.
Centimeter	cm.	Melting point	M.P.
Cubic centimeter	cc.	Meter	m.
Cubic millimeter	c.mm.	Micron (10^{-3} mm. $= 10^{-6}$ m.)	μ.
Cubic (other than in cc. and		Millimicron (10^{-6} mm.)	mμ.
c.mm.)	cu.	Micromicron (10^{-9} mm.)	μμ.
Cycles per second	c./sec.	Microampere	μA.
Direct current	D.C.	Microgram	μg.
Electromotive force	E.M.F.	Micromilligram	μmg.
Fahrenheit	F.	Micromicrogram (10^{-12} gram)	μμg.
Farad	F.	Microfarad	μF.
Figure	Fig.	Microhenry	μH.
Fractions	0.1	Micromicrofarad	μμF.
Gamma (10^{-3} mg.)	γ	Microsecond	μsec.
Gram	g.	Microvolt	μV.
Gravity, accleration due to	*g*	Milliampere	mA.

* Compiled from accepted international conventions.

Milligram	mg	Page, pages	p pp
Millihenry	mH	per cent	per cent
Milliliter	ml	Potential difference	P D
Millimeter	mm	Revolutions per minute	rev /min
Millimicron	mμ	Second (time)	sec
Millisecond (sigma)	msec	Sigma (10⁻³ sec)	σ
Millivolt	mV	Specific gravity	sp gr
Milliwatt	mW	Specific heat	sp ht
Minute (time)	min	Square (*e g* sq cm)	sq
Molar	*M*	Temperature (in table head	temp
Molecule or Molecular	mol	ings)	
Normal (of solution)	*N*	Time	a m , p m
1/10th normal	0 1 *N*	Venous pressure	v p
1/100th normal	0 01 *N*	Volume	vol
Normal temperature and	N T P	Volt	V
pressure		Watt	W
Observed (in table headings)	obs	Wave length	λ
Ohm	Ω or O		

III LIST OF PERIODICALS AND THEIR ABBREVIATIONS

*The abbreviations follow those of the World list of scientific periodicals**

PERIODICAL	ABBREVIATION
Abhandlungen der Kgl Sachsischen Gesellschaft (Akademie der Wissenschaften Math -Phys Kl) Leipzig	Abh sachs Ges (Akad) Wiss
Acta chirurgica Scandinavica	Acta chir scand
Acta medica Scandinavica	Acta med scand
Acta obstetrica et gynecologica Scandinavica	Acta obstet gynec scand
Acta opthalmologica	Acta ophthal , Kbh
Acta oto laryngologica	Acta oto laryng Stockh
Acta psychiatrica et neurologica	Acta psychiat , Kbh
Acta Scholae medicinalis Universitatis Imperialis in Kioto	Acta Sch med Univ Kioto
Alexander und Marburgs, Handbuch der Neurologie des Ohres	Handb Neurol Ohres
American Journal of Anatomy	Amer J Anat
American Journal of Diseases of Children	Amer J Dis Child
American Journal of Hygiene	Amer J Hyg
American Journal of the Medical Sciences	Amer J med Sci
American Journal of Obstetrics and Gynaecology	Amer J Obstet Gynaec
American Journal of Pathology	Amer J Path
American Journal of Pharmacy	Amer J Pharm
American Journal of Physiology	Amer J Physiol
American Journal of Psychiatry	Amer J Psychiat
American Journal of Psychology	Amer J Psychol
American Journal of Tropical Diseases	Amer J trop Dis
Anatomical Record	Anat Rec
Anatomischer Anzeiger	Anat Anz
Annalen der Chemie	Liebigs Ann
Annalen der Physik	Ann Phys , Lpz
Annales de Chimie (et de Physique)	Ann Chim (Phys)
Annales de l'Institut Pasteur	Ann Inst Pasteur
Annales de Médecine	Ann Méd

Annales de Physiologie et de Physicochimie	Ann. Physiol. Physicochim. biol.
Annales de Physique	Ann. Phys., Paris
Annales de la Société Royale des sciences médicales et naturelles de Bruxelles	Ann. Soc. Sci. méd. nat. Brux.
Annali d'Igiene (sperimentale)	Ann. Igiene (sper.)
Annals of Applied Biology	Ann. appl. Biol.
Annals of Internal Medicine	Ann. intern. Med.
Arbeiten aus d. neurologischen Institut (Inst. f. Anatomie u. Physiologie d. Zentralnervensystems) an der Wiener Universität	Arb. neurol. Inst. (Inst. Anat. Physiol. ZentNerv.) Univ. Wien
Arbeitsphysiologie	Arbeitsphysiologie
Archiv für Anatomie und Pharmakologie	Arch. Anat. Pharmak.
Archiv für Anatomie und Physiologie	Arch. Anat. Physiol., Lpz.
Archiv für Augenheilkunde	Arch. Augenheilk.
Archiv für Entwicklungsmechanik der Organismen	Arch. EntwMech. Org.
Archiv für experimentelle Pathologie und Pharmakologie	Arch. exp. Path. Pharmak.
Archiv für die gesamte Physiologie	Pflüg. Arch. ges. Physiol.
Archiv für Gynaekologie	Arch. Gynaek.
Archiv für Hygiene	Arch. Hyg., Berl.
Archiv für mikroskopische Anatomie	Arch. mikr. Anat.
Archiv für Ophthalmologie	v. Graefes Arch. Ophthal.
Archiv für pathologische Anatomie	Virchows Arch.
Archiv für Psychiatrie und Nervenkrankheiten	Arch. Psychiat. Nervenkr.
Archiv für Verdauungskrankheiten	Arch. VerdauKr.
Archives de Biologie	Arch. Biol., Paris
Archives of Internal Medicine	Arch. intern. Med.
Archives internationales de Pharmacodynamie (et de Thérapie)	Arch. int. Pharmacodyn.
Archives internationales de Physiologie	Arch. int. Physiol.
Archives italiennes de Biologie	Arch. ital. Biol.
Archives de médecine expérimentale et d'anatomie pathologique	Arch. Méd. exp.
Archives néerlandaises de Physiologie	Arch. néerl. Physiol.
Archives de Neurologie	Arch. Neurol., Paris
Archives of Neurology and Psychiatry, Chicago	Arch. Neurol. Psychiat., Chicago
Archives of Neurology and Psychiatry, London	Arch. Neurol. Psychiat., Lond.
Archives d'Ophtalmologie	Arch. Ophtal., Paris
Archives of Ophthalmology	Arch. Ophthal., N.Y.
Archives de Physiologie normale et pathologique	Arch. Physiol. norm. path.
Archives des Sciences biologiques	Arch. Sci. biol.
Archivio di farmacologia sperimentale e scienze affini	Arch. Farmacol. sper.
Archivio di Fisiologia	Arch. Fisiol.
Archivio per le Scienze mediche	Arch. Sci. med.
Association for Research in Nervous and Mental Disease Publications	Res. Publ. Ass. nerv. ment. Dis.
Atti della R. Accademia dei Lincei (Memorie)	Mem. Accad. Lincei
Atti della R. Accademia dei Lincei (Rendiconti)	R.C. Accad. Lincei
Atti della R. Accademia delle Scienze di Torino	Atti Accad. Torino
Atti del R. Istituto veneto di scienze, lettere ed arti	Atti Ist. veneto
Atti della Società lombarda di Scienze mediche e biologiche	Atti Soc. lombarda Sci. med. biol.
Australian Journal of Experimental Biology and Medical Science	Aust. J. exp. Biol. med. Sci.
Beiträge zur chemischen Physiologie und Pathologie	Beitr. chem. Physiol. Path.
Beiträge zur Geburtshilfe und Gynäkologie	Beitr. Geburtsh. Gynäk.
Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie	Beitr. path. Anat.
Bericht der deutschen chemischen Gesellschaft	Ber. dtsch. chem. Ges.

Bericht über die gesamte Physiologie und experimentelle Pharmakologie	Ber. ges. Physiol.
Berliner klinische Wochenschrift	Berl. klin. Wschr.
Bethes Handbuch der normalen und pathologischen Physiologie	Handb. norm. path. Physiol.
Bibliothek for Laeger	Bibl. Laeger
Biochemical Journal	Biochem. J.
Biochemische Zeitschrift	Biochem. Z.
Biological Bulletin of the Marine Biological Laboratory, Wood's Hole	Biol. Bull. Wood's Hole
Biological Reviews	Biol. Rev.
Biologisches Zentralblatt	Biol. Zbl.
Bolletino della Società italiano di biologia sperimentale	Boll. Soc. ital. Biol. sper.
Brain	Brain
British Journal of Children's Diseases	Brit. J. Child. Dis.
British Journal of Experimental Biology	Brit. J. exp. Biol.
British Journal of Experimental Pathology	Brit. J. exp. Path.
British Journal of Ophthalmology	Brit. J. Ophthal.
British Journal of Psychology	Brit. J. Psychol.
British Medical Journal	Brit. med. J.
Bulletin de l'Académie de Médecine de Belgique	Bull. Acad. Méd. Belg.
Bulletin de l'Académie de Médecine de Paris	Bull. Acad. Méd. Paris
Bulletin biologique de la France et de la Belgique	Bull. biol.
Bulletin of the Neurological Institute of New York	Bull. neurol. Inst. N.Y.
Bulletin of the New York Academy of Medicine	Bull. N.Y. Acad. Med.
Bulletin et mémoires de la Société anatomique de Paris	Bull. Soc. anat. Paris
Bulletin et mémoires de la Société médicale des hôpitaux de Paris	Bull. Soc. méd. Hôp. Paris
Bulletin de la Société de Chimie biologique	Bull. Soc. Chim. biol. Paris
Bumke und Foersters Handbuch der Neurologie	Bumke u. Foersters Handb. Neurol.
Canadian Medical Association Journal	Canad. med. Ass. J.
Carnegie Institution of Washington Publications	Publ. Carneg. Instn.
Cellule	Cellule
Chemical Abstracts	Chem. Abstr.
Chemische Zeitschrift	Chem. Z.
Chinese Journal of Physiology	Chin. J. Physiol.
Chinese Journal of Physiology. Report Series	Chin. J. Physiol. Rep. Ser.
Clinical Science	Clin. Sci.
Cold Spring Harbor Monographs	Cold Spr. Harb. Monogr.
Comptes rendus hebdomadaires des séances de l'Acad. des Sciences	C.R. Acad. Sci., Paris
Comptes rendus hebdomadaires des séances et mémoires de la Société de Biologie	C.R. Soc. Biol., Paris
Confinia Neurologica	Conf. neurol.
Congrès international de neurologie, de psychiatrie (et de psychologie)	Congr. int. Neurol. Psychiat. (Psychol.)
Deutsche medizinische Wochenschrift	Dtsch. med. Wschr.
Deutsche Zeitschrift für Nervenheilkunde	Dtsch. Z. Nervenheilk.
Encéphale	Encéphale
Endocrinology	Endocrinology
Endokrinologie	Endokrinologie
Ergebnisse der Physiologie	Ergebn. Physiol.
Fermentforschung	Fermentforschung
Folia anatomica japonica	Folia anat. japon.

Folia haematologica	Folia haemat.
Folia neuro-biologica. Leipzig	Folia neuro-biol. Lpz.
Fortschritte der Neurologie und Psychiatrie	Fort. Neur. Psychiat.
Gegenbaurs morphologisches Jahrbuch	Gegenbaurs Jb.
Giornale di biologia e medicina sperimentale	G. Biol. Med. sper.
Graefes Archiv	v. Graefes Arch. Ophthal.
Guy's Hospital Reports	Guy's Hosp. Rep.
Haematologica	Haematologica
Handbuch der Biochemie (Oppenheimer)	Handb. Biochem. Berl.
Handbuch der biochemischen Arbeitsmethoden	Handb. boichem. ArbMeth.
Handbuch der Neurologie des Ohres (Alexander and Marburg)	Handb. Neurol. Ohres
Handbuch der Neurologie (Bumke und Foerster)	Bumke u. Foersters Handb. Neurol.
Handbuch der Neurologie (Lewandowsky)	Lewandowskys Handb. Neurol.
Handbuch der normalen und pathologischen Physiologie (Bethe)	Handb. norm. path. Physiol.
Handbuch der Physiologie (Hermann)	Handb. Physiol.
Handbuch der vergleichenden Physiologie	Handb. vergl. Physiol.
Harvey Lectures	Harv. Lect.
Heart	Heart
Hermanns Handbuch der Physiologie	Handb. Physiol.
Hoppe-Seylers Zeitschrift f. physiologische Chemie	Hoppe-Seyl. Z.
Indian Journal of Medical Research	Ind. J. med. Res.
International Congress of Experimental Psychology	Int. Congr. exp. Psychol.
International Physiological Congress	Int. physiol. Congr.
Internationale Monatsschrift f. Anatomie und Physiologie	Int. Mschr. Anat. Physiol.
Jahrbuch für Psychiatrie und Neurologie	Jb. Psychiat. Neurol.
Jahresbericht Physiologie und experimentelle Pharmakologie	Jber. Physiol. exp. Pharm.
Japanese Journal of Medical Sciences	Jap. J. med. Sci.
Part 2. Biochemistry	Biochem.
Part 3. Biophysics	Biophys.
Johns Hopkins Hospital Bulletin	Johns Hopk. Hosp. Bull.
Johns Hopkins Hospital Reports	Johns Hopk. Hosp. Rep.
Journal of the American Chemical Society	J. Amer. chem. Soc.
Journal of the American Medical Association	J. Amer. med. Ass.
Journal de l'Anatomie et de la Physiologie	J. Anat., Paris
Journal of Anatomy	J. Anat., Lond.
Journal of Bacteriology	J. Bact.
Journal belge de Neurologie et Psychiatrie	J. belge Neurol. Psychiat.
Journal of Biochemistry	J. Biochem.
Journal of Biological Chemistry	J. biol. Chem.
Journal of Biophysics	J. Biophys., Tokyo
Journal of Cellular and Comparative Physiology	J. cell. comp. Physiol.
Journal of Clinical Investigation	J. clin. Invest.
Journal of Comparative Neurology	J. comp. Neurol.
Journal of Comparative Psychology	J. comp. Psychol.
Journal of Experimental Biology	J. exp. Biol.
Journal of Experimental Medicine	J. exp. Med.
Journal of Experimental Psychology	J. exp. Psychol.
Journal of Experimental Zoology	J. exp. Zool.
Journal of General Physiology	J. gen. Physiol.
Journal of General Psychology	J. gen. Psychol.
Journal of Genetics	J. Genet.
Journal of Hygiene	J. Hyg., Camb.

Journal of Infectious Diseases	J infect Dis
Journal of Laboratory and Clinical Medicine	J Lab clin Med
Journal of the Marine Biological Association	J Mar biol Ass U K
Journal of Medical Research	J med Res
Journal of Mental Science	J ment Sci
Journal of Metabolic Research	J metab Res
Journal of Nervous and Mental Diseases	J nerv ment Dis
Journal de Neurologie	J Neurol Brux
Journal of Neurology and Psychopathology	J Neurol Psychopath
Journal of Neurophysiology	J Neurophysiol
Journal of Nutrition	J Nutrit
Journal of Obstetrics and Gynaecology	J Obstet Gynaec
Journal of the Optical Society of America	J opt Soc Amer
Journal of Pathology and Bacteriology	J Path Bact
Journal de Pharmacie et de Chimie	J Pharm Chim , Paris
Journal of Pharmacology and Experimental Thera peutics	J Pharmacol
Journal de Physiologie et de Pathologie genérale	J Physiol Path gén
Journal of Physiology	J Physiol
Journal fur Psychologie und Neurologie	J Psychol Neurol , Lpz
Journal de Psychologie normale et pathologique	J Psychol norm path
Journal of Psychology	J Psychol , Provincetown
Journal of the Royal Microscopical Society	J R micr Soc
Journal of Scientific Instruments	J sci Instrum
Journal of Washington Academy of Sciences	J Wash Acad Sci
Klinische Monatsblatter fur Augenheilkunde	Klin Mbl Augenheilk
Klinische Wochenschrift	Klin Wschr
Kolloidzeitschrift	Kolloidzschr
Lancet	Lancet
Lewandowskys Handbuch der Neurologie	Lewandowskys Handb Neurol
Liebigs Annalen der Chemie	Liebigs Ann
Medical Journal of Australia	Med J Aust
Medical Journal of South Africa	Med J S Afr
Medical Science Abstracts and Reviews	Med Sci
Medico Chirurgical Transactions	Med chir Trans
Mémoires couronnés et autres mémoires p p l Acad émie R de médecine de Belgique	Mém cour Acad Med Belg
Mitteilungen aus den Grenzgebieten der Medizin und Chirurgie	Mitt Grenzgeb Med Chir
Monatsschrift fur Kinderheilkunde	Mschr Kinderheilk
Monatsschrift fur Ohrenheilkunde u Laryngologie	Mschr Ohrenheilk
Monatsschrift fur Psychiatrie und Neurologie	Mschr Psychiat Neurol
Monitore zoologico italiano	Monit zool ital
Morgagni	Morgagni
Morphologisches Jahrbuch	Morph Jb
Munchener medizinische Wochenschrift	Munch med Wschr
Nature	Nature, Lond
Naturwissenschaften	Naturwissenschaften
Nederlandsch Tijdschrift voor Geneeskunde	Ned Tijdschr Geneesk
Nervenarzt	Nervenarzt
Neurological Bulletin	Neurol Bull
Neurologisches Zentralblatt	Neurol Zbl
Névraxe	Nevraxe
New England Journal of Medicine	New Engl J Med
Oppenheimers Handbuch der Biochemie	Handb Biochem , Berl

Pflügers Archiv für die gesamte Physiologie	Pflüg. Arch. ges. Physiol.
Philosophical Magazine	Phil. Mag.
Philosophical Transactions of the Royal Society	Philos. Trans.
Physikalische Zeitschrift	Phys. Z.
Physiological Abstracts	Physiol. Abstr.
Physiological Reviews	Physiol. Rev.
Policlinico	Policlinico
Presse médicale	Pr. méd.
Proceedings of the National Academy of Sciences, Washington	Proc. nat. Acad. Sci., Wash.
Proceedings of the Physiological Society	Proc. Physiological Soc.
Proc. of the Royal Academy of Sciences Amsterdam	Proc. Acad. Sci. Amst.
Proceedings of the Royal Dublin Society	Sci. Proc. R. Dublin Soc.
Proceedings of the Royal Society	Proc. roy. Soc.
Proceedings of the Royal Society of Canada	Proc. roy. Soc. Can.
Proceedings of the Royal Society of Edinburgh	Proc. roy. Soc. Edinb.
Proceedings of the Royal Society of Medicine	Proc. R. Soc. Med.
Proceedings of the Society of Experimental Biology and Medicine	Proc. Soc. exp. Biol., N.Y.
Proceedings of the Zoological Society London	Proc. zool. Soc. Lond.
Psychiatrische en neurologische bladen, Amsterdam	Psychiat. neurol. Bl., Amst.
Psychiatric Quarterly	Psychiat. Quart.
Psychological Monographs	Psychol. Monogr.
Psychological Rev.	Psychol. Rev.
Pubblicazioni della Stazione zoologica di Napoli	Pubbl. Staz. zool. Napoli
Quarterly Journal of Experimental Physiology	Quart. J. exp. Physiol.
Quarterly Journal of Medicine	Quart. J. Med.
Quarterly Journal of Microscopical Science	Quart. J. micr. Sci.
Quarterly Journal of Pharmacy and Allied Sciences	Quart. J. Pharm.
Quarterly Review of Biology	Quart. Rev. Biol.
Rendiconti della R. Accademia dei Lincei	R.C. Accad. Lincei
Reports of British Association	Rep. Brit. Ass.
Research Publications Association for Research in Nervous and Mental Disease	Res. Publ. Ass. nerv. ment. Dis.
Revue française d'endocrinologie	Rev. franç. Endocrin.
Revue neurologique	Rev. neurol.
Riforma Medica	Rif. med.
Rivista di biologia	Riv. biol.
Science	Science
Science Abstracts	Sci. Abstr.
Scientific Proceedings of the Royal Dublin Society	Sci. Proc. R. Dublin Soc.
Schweizer Archiv für Neurologie und Psychiatrie	Schweiz. Arch. Neurol. Psychiat.
Sitzungsberichte der Akademie der Wissenschaften in Wien	S.B. Akad. Wiss. Wien
Abt. III Anatomie und Physiologie	Anat. Physiol.
Sitzungsberichte der Kgl. preussischen Akademie der Wissenschaften z. Berlin	S. B. preuss. Akad. Wiss.
Skandinavisches Archiv für Physiologie	Skand. Arch. Physiol.
South African Journal of Science	S. Afr. J. Sci.
Special Report Series Medical Research Council London	Spec. Rep. Ser. med. Res. Coun., Lond.
Sperimentale. Archivio di biologia normale e patologica	Sperimentale
Stanford University Publications—University Series Biological Sciences Medical Sciences	Stanford Univ. Publ. biol. Sci. med. Sci.
Surgery, Gynecology and Obstetrics	Surg. Gynec. Obstet.

Tabulae biologicae	Tabul biol , Berl
Tohoku Journal of Experimental Medicine	Tohoku J exp Med
Transactions of the Faraday Society	Trans Faraday Soc
Transactions of the Optical Society	Trans opt Soc Lond
Transactions of the Royal Society of Canada	Trans Roy Soc Can
Transactions of the Royal Society of Edinburgh	Trans Roy Soc Edinb
Transactions of the Royal Society of South Africa	Trans Roy Soc S Afr
Transactions of the Zoological Society	Trans zool Soc Lond
Travaux du Laboratoire des recherches biologiques de l'Université de Madrid	Trab Lab Invest biol Univ Madr
Ugeskrift for Laeger	Ugeskr Laeg
University of California Publications	Univ Calif Publ
Verhandelingen der (Kon) Akad van Wetenschappen	Verh Akad Wet , Amst
Verhandelungen der anatomischen Gesellschaft	Verh anat Ges Jena
Verhandelungen der Deutschen Gesellschaft der innere Medizin	Verh dtsch Ges inn Med
Verhandelungen der Gesellschaft deutscher Nervenarzte	Verh Ges dtsch Nervenarz
Virchows Archiv fur pathologische Anatomie und Physiologie und fur klinische Medizin	Virchows Arch
Wiener klinische Wochenschrift	Wien klin Wschr
Wiener medizinische Wochenschrift	Wien med Wschr
Zeitschrift fur allgemeine Physiologie	Z allg Physiol
Zeitschrift fur Augenheilkunde	Z Augenheilk
Zeitschrift fur Biologie	Z Biologie
Zeitschrift fur experimentelle Pathologie und Therapie	Z exp Path Ther
Zeitschrift fur die gesamte Anatomie	Z ges Anat
Zeitschrift fur die gesamte experimentelle Medizin	Z ges exp Med
Zeitschrift fur die gesamte Neurologie und Psychiatrie	Z ges Neurol Psychiat
Zeitschrift fur Hygiene und Infektionskrankheiten	Z Hyg InfektKr
Zeitschrift fur Immunitatsforschung	Z ImmunForsch
Zeitschrift fur Kinderheilkunde	Z Kinderheilk
Zeitschrift fur klinische Medizin	Z klin Med
Zeitschrift fur Kreislaufforschung	Z KreislForsch
Zeitschrift fur die medizinische Wissenschaften	Z med Wiss
Zeitschrift fur mikroskopisch anatomische Forschung	Z mikr anat Forsch
Zeitschrift fur physikalische Chemie	Z phys Chem
Zeitschrift fur physiologische Chemie	Hoppe Seyl Z
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THE COURSE OF RECOVERY OF THE SPINAL CORD FROM ASPHYXIA

A. van HARREVELD AND GEORGE MARMONT

*William G. Kerckhoff Laboratories of the Biological Sciences,
California Institute of Technology, Pasadena, California*

INTRODUCTION

THE EFFECT of the onset of asphyxia on the spinal reflexes has been investigated many times, recently by Montgomery and Luckhardt (1929), by King, Garrey, and Bryan (1932) and by Porter, Blair and Bohmfalk (1938) During the development of asphyxia a period of increased reflex activity, which precedes the abolition of the reflexes, has been usually described In this paper experiments will be described in which the spinal cord of cats has been asphyxiated for various periods, the animals were observed up to three weeks after the experiment, and the recovery of reflex activity after asphyxia was especially studied

The spinal cord is a difficult organ to asphyxiate by operative methods because it is abundantly supplied with blood vessels Tureen (1936) found it necessary to clamp the thoracic aorta to obtain a complete anoxemia of the lower part of the spinal cord He succeeded in keeping only alive cats that had been subjected to this procedure for periods up to 15 min In our preliminary attempts to asphyxiate the cord such operative methods were tried The cord itself was ligated in the lower thoracic region to stop the blood supply from the spinal arteries The aorta was clamped just below the point where the renal arteries branch off, thus preventing damage to the kidneys The coeliac, superior mesenteric, adreno lumbar and lumbar arteries, all of which branch off between the aorta clamp and the diaphragm, were clamped also As an extra precaution the gut was clamped with intestinal forceps The animal was given ether anaesthesia during the experiment Many of these animals in which the cord had been asphyxiated for 45–50 min were observed for several weeks following the operation

The results in cats subjected to this complicated operation were variable The prime cause of the variability was probably that the blood vessels to the body muscles anastomose along the whole length of the body and therefore the asphyxia of the cord was often incomplete The other disadvantage was that the entire posterior half of the body was asphyxiated This made it difficult to decide whether the symptoms developing after the operation were entirely the result of asphyxia of the cord The mortality was high These preliminary experiments agreed in the main with those now to be described

METHOD

The principle of this second method, used in obtaining the data here reported, was employed by Cushing in 1902 It involves raising the pressure inside the vertebral canal

to a higher level than the blood pressure, thereby occluding the blood vessels supplying its contents. Five days previous to the planned asphyxia the vertebral canal was aseptically opened and the cord ligated at Th9 to Th10, without opening the dura. This made it possible to apply pressure to the lower half of the cord and to avoid compressing the anterior part of the nervous system. The five day period between the operation and the asphyxia allowed the spinal cord to recover from shock. A thick injection needle was inserted intradurally between L6 and L7, and through it sterile Ringer-Locke solution was brought into the subdural space under a pressure of 23–25 cm. Hg. To exclude oxygen from the solution, pure nitrogen was bubbled through it previous to the experiment and was also used to produce and maintain the pressure. Under this pressure the physiological
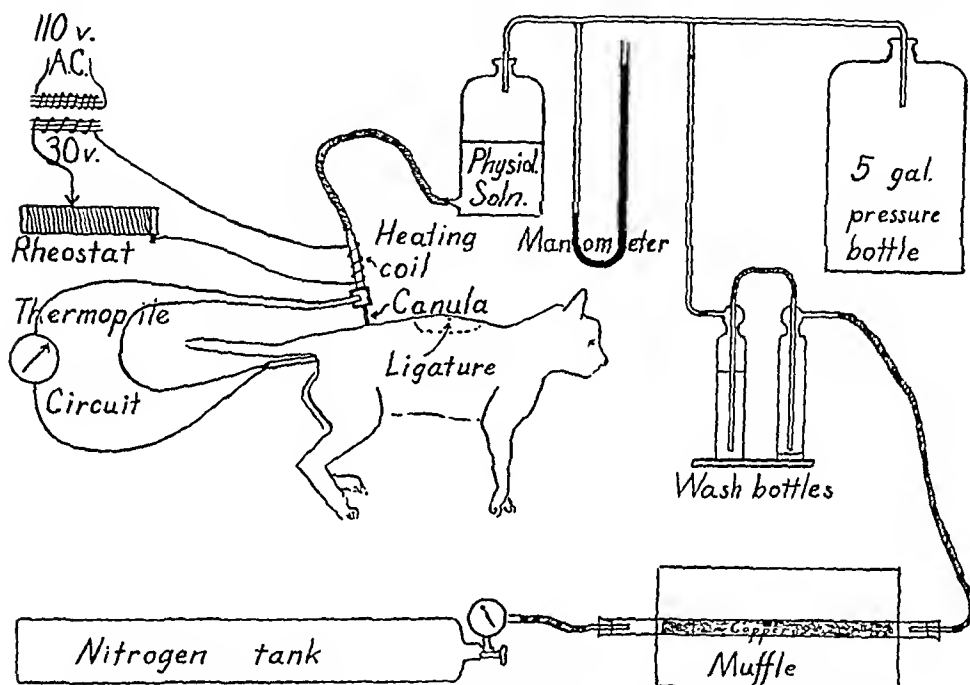

FIG. 1. Diagram of the apparatus used in asphyxiating the cord. See text for a description of the procedure used.

solution disappeared from the vertebral canal at a rate of 1 to 4 cc. per min.; this lost fluid was replaced by physiological solution from a reservoir.

To keep the temperature of the cord from falling, which would depress the metabolism of the cord and introduce a variable factor, the solution was maintained at body temperature by passing it through a tube around which a heating coil was wound. The current through this coil was so adjusted that a galvanometer contained in a thermopile circuit measuring the temperature difference between the solution in the needle and cat's rectum did not show a deflection (Fig. 1). In a number of cases the rectal temperature was measured during the experiment; it was always found to be normal. Fig. 1 is a diagram of the set-up. Controls showed that this pressure method really prevents blood from reaching the lower half of the cord. While the cord was under pressure, 10 cc. of India ink were injected intravenously. The animal was then killed and the cord fixed with formalin. The pressure was maintained in the caudal part of the dural cavity during fixation by changing the physiological solution to formalin under pressure. An abundance of carbon particles was present in the blood vessels of the cord in sections taken cranial to the ligature, none in the caudal cord.

The advantages of this method are obvious. The spinal cord only is asphyxiated, the

peripheral part of the reflex apparatus receiving its normal blood supply, and thus changes in the reflexes can only be ascribed to changes in the central part of the arc It might be objected that this procedure subjects the spinal cord not only to asphyxia but also to pressure. A small pressure of 23–25 cm Hg acting equally on every part of the cord does not produce any injury, as demonstrated by the innocuousness of much higher pressure in caissons The sudden change from high to normal pressure at the inter-vertebral foramina, where the spinal nerves pass, might produce mechanical injury to the spinal nerves, but this is ruled out by the fact that Marchi preparations of the dorsal roots of an animal in which the cord was asphyxiated for 75 min did not show any degeneration 20 days after the experiment

RESULTS

The spinal cord was asphyxiated by pressure for periods ranging from 25 to 75 min. Before beginning the experiment the reflexes and tone in the hind legs were tested. They were usually typical for cats made spinal 5 days previously; the knee jerk was brisk, there was a strong flexor reflex with appreciable crossed extension, and the tone was low and usually on the flexor side. The presence of the achilles tendon reflex was variable. When pressure had been applied to the cord, the knee jerk was tested frequently as a measure of the progress of asphyxia. It was usually abolished after 1–1.5 min. of pressure, decidedly quicker than when other methods are used to produce asphyxia. The knee jerk was increased for a short period before disappearing, and in some experiments tonic and clonic hind leg movements were observed shortly after beginning the asphyxia. These movements usually stopped within a minute; in a few cats, however, a slight tonic extensor contraction lasted as long as 10 min.

When the animal was restive, light ether anaesthesia was used only after pressure had been applied and was always discontinued at least 10 min. before the end of the experiment. Often no anaesthetic was necessary, merely stroking the cat sufficing to keep it quiet. The asphyxiated part of the cord was thus never narcotized at any time. After releasing the pressure the return of reflexes and tone in the hind legs was carefully followed several times an hour, then hourly, and, after a few days, once daily. The first sign of returning reflex activity was either a slight tone in the triceps or quadriceps, or a weak achilles tendon reflex or knee jerk. The time required for the return of reflexes and the further behavior of the cord varied with the duration of asphyxia. We shall, therefore, discuss separately each group of animals subjected to a like period of asphyxia.

In two cats pressure was applied for 75 min. Neither animal gave any certain sign of reflex activity in the hind legs during the three weeks of survival. In 5 cats the spinal cord was asphyxiated for 65 min. The times of appearance, and subsequent disappearance, of tone and reflexes are given in Table 1. In one animal (65B) neither tone nor reflexes were observed at any time. In one (65A) which gave tendon reflexes, tone reached a maximum 5 hr. after asphyxia and then declined, both tone and reflexes disappearing completely within 24 hr. The other three cats showed tone only, which appeared some hours after the asphyxia and disappeared completely a few hours later. Tone was observed only in the extensor muscles. After

the disappearance of tone (and reflexes in cat 65A) no reflexes of any kind could be elicited during the period of survival, in some cases as long as three weeks.

The cord was asphyxiated for 55 min. in 5 animals. Tone in the extensor muscles and tendon reflexes returned in all, but only temporarily (see

Table 1. *The appearance and disappearance of tone and reflexes in cats in which the spinal cord was asphyxiated. The cords indicated as 25A, 25B etc. were asphyxiated for 25 min.; those indicated as 35A, 35B etc. were asphyxiated for 35 min. etc. An asterisk means that the reflex or tone remained until death.*

Cat	Tone Begin End (hr.)		Knee jerk Begin End (hr.)		Ach. t. refl. Begin End (hr.)		Flex. refl. Begin End (hr.)		Tail refl. Begin End (hr.)		Period of observation (days)
25A	1.5	*	0.5	*	0.5	*	0.5	*	1	*	21
25B	2	*	1	*	1	*	2.5	*	8	*	2
25C	0.3	*	1	*	0.5	*	3.5	*	1.5	*	3
25D	2	*	0.5	*	9	*	6	*	1	*	4
25E	0.3	*	1	*	—		4	*	4	*	20
35A	1	*	0.5	*	0.5	*	9	*	9	*	20
35B	2	*	4	*	1.5	*	24	*	24	*	21
35C	1	*	4	*	4	*	24	*	7	*	18
35D	1.5	*	1	*	1.5	*	7	*	7	*	13
35E	1	72	1	*	1.5	24	—		4.5	*	20
45A	0.2	*	0.2	*	0.3	*	1.5	14	2.5	14	10
45B	1.5	*	0.5	*	1.5	4	—		—		3
45C	6	*	4	*	6	*	—		—		16
45D	2	48	1	48	2	24	10	24	5	48	6
45E	1	32	2	32	2	12	—		7	10	8
55A	2	6	2	5	2	5	—		—		14
55B	2.5	48	2	48	2.5	32	—		—		10
55C	1.5	48	1.5	32	1	24	—		—		8
55D	1.5	32	4.5	48	3	32	—		—		21
55E	1.5	14	8	24	—		—		—		4
65A	4	9	4	24	2.5	24	—		—		2
65B	—		—		—		—		—		7
65C	2	5	—		—		—		—		20
65D	1.5	3.5	—		—		—		—		3
65E	1.5	5.5	—		—		—		—		20

Table 1). The extensor tone became so high in some cats that the tendon reflexes were masked. Before this powerful tone developed the tendon reflexes themselves were quite brisk. Here we encountered a phenomenon that will be met a number of times in this paper: asphyxia caused a marked increase of the reflex excitability of the cord not only at the beginning of asphyxia but for long periods after restoration of the circulation. Another sign of the increased excitability was the clonus observed in these animals either following a knee jerk or achilles tendon reflex or "spontaneously" when the leg was in certain positions. The maximum tonus in cats 55A, B

and E was comparable to that in the fore legs during moderate decerebrate rigidity; that in cat 55C was markedly higher. Neither reflexes nor tone remained longer than 48 hr. after the asphyxia in any case, but after 5 to 6 days a moderate extensor tone redeveloped in three of the cats. In one (55D) even a small but easily elicited knee jerk reappeared. No flexor or tail reflexes were observed at any time after asphyxia.

The next group of 5 animals was subjected to spinal pressure for 45 min. In 2 cats (45D and E) the appearance and disappearance of tone and reflexes was quite similar to that in the 55-minute group, except that a slight flexor reflex and slight movements on pinching the tail appeared temporarily, as noted in Table 1. The other animals differed in that the tendon reflexes and tone remained until death. Indeed, in cats 45A and C, the extensor tone became so great that it could be compared only to a well developed rigor mortis; the extended legs easily supported the weight of the body during the whole survival period (10 and 16 days). In all cases strong tendon reflexes and clonus were observed at some period following asphyxia until they disappeared or became masked by the intense tonus.

FIG. 2. Photograph of cat 35A, in which the spinal cord was asphyxiated for 35 min. (12 days after asphyxia). The animal is standing unassisted; note the extreme stretching of the hind legs which easily carry the body weight.

In the 35-minute asphyxia group of 5 cats (Table 1), the typical spinal reflexes returned and remained (except that the crossed extension reflex was only observed in cat 35B). The tone in the extensor muscles usually became very high. Fig. 2 is a photograph of cat 35A showing that the strongly extended hind legs can easily carry the body weight. In some animals the strong extensor tone was present during the first two days after asphyxia and then disappeared (35D and E); in others it was only moderate for the first two days, but increased greatly after that (35A and B), and remained during the period of observation, 20 and 21 days. In one animal, the tone did not become high at any time (35C). Vigorous tendon reflexes and clonus were observed in all cases. In cat 35C the tail reflexes became so extremely brisk that the slightest touch of the hair would evoke movements.

Pressure was applied for 25 min. to the final group of 5 animals (Table 1). Tone and tendon reflexes returned between 20 min. and 2 hr. Tail reflexes and the flexion reflex, usually with crossed extension, developed in all 5 animals within 9 hr. The tone was slight to moderate, except that in cat 25E it became intense after the first two days and remained so until after the

seventh day. Cat 25B developed a preponderant and continuous flexor tone and cat 25D extremely brisk tail reflexes. These animals resembled normal spinal cats in many respects, though their reflexes were more vivid.

The increase of reflex excitability and tone after asphyxia

Asphyxia of the cord thus commonly resulted in increased reflex excitability and high extensor tone. After an asphyxia of 35 to 55 min. exaggerated tendon reflexes were usually present and could be evoked by the slightest tap on the tendon so long as excessive tone in the extensor muscles did not mask them. The tendon achilles reflex offered a convincing example of this heightened response. Five days after cord ligation this reflex was usually low and sometimes absent. After asphyxia it was often the first reflex to return and usually became strong after some hours. The marked clonus in the extensor muscles frequently observed after asphyxia is another sign of the increased excitability. The skin reflexes were seldom marked, but very readily elicited tail reflexes were observed in one 25-minute and one 35-minute cat.

The question arises whether the high extensor tone is the result of a real reflex or whether the motor neurons discharge spontaneously. There is some reason to believe that the latter event could occur. Fibrillar contractions of the toes and muscles of the leg were often seen for a few hours after asphyxia; this type of contraction is usually considered evidence of damage to the anterior horn cells. These fibrillations always disappeared shortly, however, while the tone could continue for weeks.

Direct proof that the tone was of reflex origin was provided by its decrease after dorsal root section. The cord was carefully exposed in 7 animals showing marked tone and the dorsal roots transected on one side from L5 down. The following animals were used: 55-minute group, cat 55D after 21 days; 45-minute group, cat 55A after 10 days, and a special animal, not included in the table, which developed a high tone in 6 hours when the roots were cut; 35-minute group, cats 35A, C and E after 23, 18 and 20 days respectively; 25-minute group, cat 25E after 20 days. The result was always that the tone on the transected side either disappeared or diminished greatly; on the opposite side the tone remained. As in decerebrate rigidity, the post-asphyxial tone in the extensor muscles can persist for days and even weeks and it is located in the extensor muscles of the large joints with the toe muscles not participating.

The time course of tonus changes falls into three categories. In type A, seen after 35 to 65 min. asphyxia, tone appears a few hours after asphyxia, sometimes reaches a considerable height, and disappears again within 48 hr. Five to six days later slight tone may develop again. In type B, after 25 to 45 min. asphyxia, the tone is rather low for the first 2 days and then increases to a considerable height (comparable to rigor mortis). In type C, after 25 min. of asphyxia and in one case after 35, the tone remains low throughout.

Tureen (1936) also observed periods of high tone and increased reflex excitability in cats after short periods of asphyxia.

Histological changes

The lower spinal cords, level L6 to L7, from cats that survived the asphyxia 14 days or longer and from normals were fixed in 95 per cent alcohol, dehydrated, embedded in paraffin, sectioned and stained with toluidine blue. Even asphyxia of 25 min. caused a marked decrease in the number of nerve
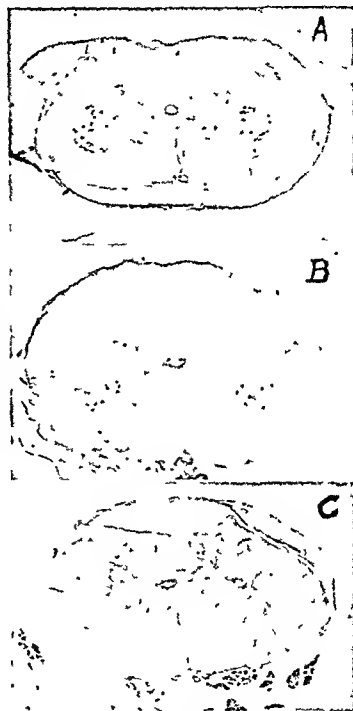

FIG 3 Photomicrographs of sections of the spinal cord taken at L6 The sections were stained with toluidine blue and photographed through a red filter A, normal cord Notice the numerous larger anterior horn cells and the great number of smaller ganglion cells in the grey matter B, preparation of cat 25E, taken about 3 weeks after a 25-minute asphyxia of the cord The anterior horn cells are less numerous Remarkable, however, is the diminution in the number of the smaller ganglion cells In this animal a high tone was temporarily observed C, preparation of the cord of cat 65C, subjected to asphyxia for 65 min 3 weeks previously Note the absence of any cell bodies.

cells, as compared with the normal, although a fair number of large motor cells remained in the anterior horn. The smaller ganglion cells in the rest of the grey substance suffered more than the large motor neurons (Fig. 3). After an asphyxia of 45 min. only a few anterior horn cells were left and the smaller ganglion cells had almost disappeared. After 55 and 65 min. only an occasional cell could be found. In many preparations the large ganglion cells in the anterior horn were counted. Average values are: normal, 33; 25-minute asphyxia, 25; 35-minute, 11; 45-minute, 6; 55-minute, 2; and after 65-minute, only 1.

DISCUSSION

Certain durations of asphyxia caused a reappearance of reflexes and tone which was only temporary, lasting less than 48 hr. Since tone and even tendon reflexes have been seen to reappear temporarily after cord asphyxia of 65 min. and since subsequent histological preparations from these animals show almost every ganglion cell killed, it must be concluded that neurons which have been so greatly damaged that they will shortly die are still able to function temporarily, with full reflex conduction maintained, including synaptic transmission. The return of tone in three 55-minute cats, and of tendon reflexes in one of these, 5 to 6 days after an initial temporary presence indicates that after this asphyxia the perikarya could not have been completely destroyed. But it may be assumed that the neurons were damaged to such an extent that certain functions required for the maintenance of conduction were temporarily abolished, to return 5 to 6 days later. Since the tone developing 5 to 6 days after asphyxia was slight, presumably a large majority of the cells was killed. After shorter asphyxia a larger number of cells survive and must maintain continuous function.

There is an increasing resistance of the reflex mechanism from skin reflexes to tendon reflexes to tone. Four of five 65-minute cats showed temporarily an appreciable tone; tendon reflexes reappeared regularly in the 55-minute animals and in one 65-minute cat. Skin reflexes reappeared regularly after 35 min. of asphyxia and twice in the 45-minute animals. This difference in resistance may be explained by differences in sensitivity of the neurons or in the number of neurons involved in the several reflex arcs. With fewer neurons in the arc the less is the chance of one dropping out, and in this respect it is significant that tendon (and tone) reflexes are supposed to have the most simple reflex arcs.

The persistence of decerebrate rigidity has caused it to be considered a release phenomenon (Bazett and Penfield, 1922). The increased tone which may persist for weeks after asphyxia might similarly be the consequence of a release. This would mean that a functional system which normally inhibits the reflex activity of the spinal cord is abolished by asphyxia, releasing the excitatory activity of the cord. It may well be a general rule that the inhibiting systems of the central nervous system are more sensitive to asphyxia than the excitatory ones, as Barcroft and Barron (1937) found for sheep embryos. The tone in a spinal animal would, then, be interpreted as an equilibrium between excitatory and inhibitory components. Asphyxia, by damaging the inhibitory component more severely than the excitatory one, shifts the equilibrium towards the excitatory side. We have further evidence that a tone inhibiting system is damaged following asphyxia. In some cases, forcible bending of a hind leg having high extensor tone was followed by a spring-like return upon release; in others by the disappearance of tone for several seconds (lengthening reaction). In these latter cases the inhibiting component was still capable of function, whereas in the former it was abolished.
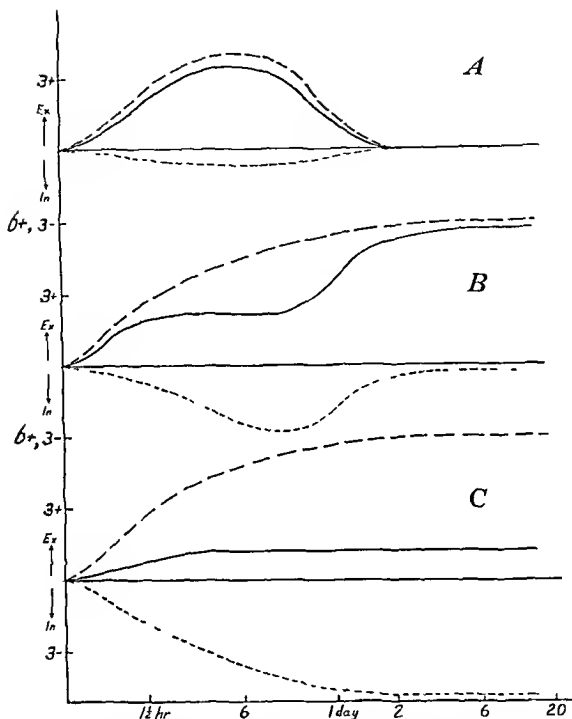
FIG 4 Diagrammatic explanation of the course of tone in the extensors of the hind leg after asphyxia designated in the text as types A, B, and C The dashed curves above each horizontal axis represent the activity of the structures involved in the excitatory component of the reflex causing the extensor tone, the dotted curves below the axis represent the activity of the structures involved in the inhibitory component of this reflex, and the algebraic sum of these two curves, representing the actual tone, is the full line appearing above each axis This full curve corresponds to the tone usually found in animals whose cords have been asphyxiated A, 65 and 55 min , B, 35 and 45 min , C, 25 min The positive ordinates represent an arbitrary scale of tone from 0 to 3, tone progresses from absolute flaccidity to that seen in a moderate decerebrate rigidity of the front legs, from this point to 6 it increases until it is comparable to rigor mortis The abscissae represent the time on a logarithmic scale

An explanation of the three types of effect of asphyxia on extensor tone is possible along these lines (Fig. 4). After long asphyxia tone reappears only temporarily, after shorter periods it remains. Presumably after long as-

phyxia the excitatory component functions temporarily, after shorter periods permanently; and the same may be assumed for the inhibitory component. This latter component has less resistance to asphyxia. A certain period of asphyxia, then, might damage the inhibitory component to such an extent that it resumes function only temporarily, while the excitatory component will remain permanently. We believe this to be the explanation of type B curve, observed after asphyxia for 25 to 45 min. For 2 days both components of the tone reflex function, resulting in a moderate tone; later the inhibitory component is lost and the more resistant excitatory component remains unopposed. After the shorter periods of asphyxia both the excitatory and inhibitory components resume their function and a moderate permanent tone results from this equilibrium (type C tone). With sufficient asphyxia to permit the excitatory component only temporary function, the inhibitory element would be severely damaged, leaving excitation more or less unopposed. Actually some of the 55-minute cats did manifest strong extensor tone (type A). After still longer asphyxia (65 min.) extensor tone remains moderate due to damage of the excitatory component. We are aware that the course of tone may be complicated by the recovery of damaged neurons 5 to 6 days after the asphyxia. In cat 25E, asphyxiated for 25 min., the drop of the high tone 7 days after the pressure may have been due to a similar recovery of the inhibiting component.

The explanation given for the high tone after asphyxia would apply also to the increased reflex excitability of the tendon reflexes. Since exaggerated tail reflexes have been observed after asphyxia, it seems that here also a normal inhibiting component was selectively damaged. The excitatory component of these skin reflexes is almost certainly more complicated than in the case of the tendon reflexes, so that the excitatory and inhibitory components probably have a more equal resistance to asphyxia and the selective abolition of the inhibitory component would be less certain. This is in keeping with the observation of Porter (1912), who did not find increased excitability of the flexor reflex during the development of asphyxia. In recent experiments, with more refined methods, however, this has been obtained in a comparable reflex (Porter, Blair, and Bohmfalk, 1938). On this interpretation the increased reflex excitability of the tendon reflexes and perhaps the convulsions which are observed during the development of asphyxia of the cord are to be explained in the same way; namely, by the differential injury of inhibiting systems. The increased reflex excitability observed during the development of asphyxia is not an excitation phenomenon, as often maintained, but, on the contrary, one of release.

SUMMARY

1. The effect of asphyxia on the lumbo-sacral cord of spinal cats was investigated, the asphyxia being produced by raising the intradural pressure above that of the arterial blood, thereby preventing blood from reaching the region involved. Asphyxia was maintained for periods of 25–75 min. and the

behavior of spinal reflexes observed during this time and for as long as 3 weeks following.

2. After long asphyxia (55 and 65 min.) tendon reflexes and tone returned for 48 hr. at the most, then disappeared. In 3 of the 55-minute cats a moderate extensor tone returned again after 5 to 6 days. After shorter periods (35 to 55 min.) these reflexes and the extensor tone returned permanently and were intense. Moderate tone and all the usual reflexes returned shortly after 25 min of asphyxia and remained.

3. The strong extensor tone is of reflex origin since transection of the dorsal roots of the lower cord abolishes it

4. Histological preparations of the cord showed that only 3 to 75 per cent of the normal number of anterior horn cells were present 14 days following the asphyxia. The number surviving diminished with increasing duration of asphyxia

5. It is concluded that the increased reflex excitability and the exaggerated tone are the result of release, the normal inhibitory systems of the cord being more damaged by asphyxia than the excitatory systems

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CORTICAL RESPONSE TO SENSORY STIMULATION UNDER DEEP BARBITURATE NARCOSIS

A. FORBES AND B. R. MORISON

From the Department of Physiology, Harvard Medical School, Boston, Mass.

IN A PREVIOUS PAPER (Derbyshire, Rempel, Forbes and Lambert, 1936) a remarkable effect was reported in the deeper stages of avertin and pento-barbital-sodium anesthesia, and in asphyxia*—an effect which differentiated the action of these agents sharply from that of ether. This was a conspicuous electric response of the cerebral cortex to stimulation of the sciatic nerve, appearing with a latency of about 40 to 80 msec. and lasting for a similar or somewhat longer period. It was found only when the cerebrum was so deeply narcotized that the record of its electrical activity afforded a relatively smooth base line as compared with that in the lighter stages of narcosis. It was subject to extremely rapid fatigue (or inhibition) since, when a rapid series of stimuli (5 or more per sec.) was applied to the sciatic nerve, this large wave, or "spike," appeared only in response to the first of the series; a rest of about 0.5 sec. was required to enable another stimulus to evoke the effect, and considerably more than a second if the effect were to attain its initial magnitude. When narcosis was so deep that the base line of the electrical record became quite smooth, it could be seen that the large excursion described above was regularly preceded by a smaller excursion whose latency (from the sciatic stimulus) was about 10 or 12 msec. The agreement of this latency with that of responses recorded in the brain-stem in previous researches (Forbes and Miller, 1922; Leese and Einarson, 1934) led to the inference that this preliminary excursion represents the arrival of the volley of afferent impulses in the cerebrum.

In the experiments of Derbyshire, Rempel, Forbes and Lambert, when the "active" (grid) lead was placed on the sensory or motor area for the hind leg and the ground lead on a cauterized area of the opposite cortex, the large "secondary" spike was regularly positive, *i.e.*, grid electrically positive to ground. In other parts of the cortex, remote from the sensorimotor area, responses of similar time relations were found, in some places grid-negative, in others diphasic. They seem to denote a widespread disturbance. In view of the regular occurrence of this response at a distinct time interval after the arrival of the afferent volley, we propose to designate it the "secondary discharge."

That in moderate narcosis no such effect appears—indeed no recognizable response to sciatic stimulation—whereas when narcosis is so deep that the cortex has become perfectly quiescent this marked response follows the

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* In a few experiments performed since the publication of that paper, the same effect has been found under Dial anesthesia. We wish to acknowledge the generous donation of Dial by Ciba Pharmaceutical Products, Inc.

stimulus with almost unfailing regularity, seems paradoxical. That no such effect was found in deep ether anesthesia suggested the conclusion that ether anesthesia when deep blocks the afferent paths leading to the cortex, whereas the hypnotic drugs (pentobarbital and avertin) stop the spontaneous activity of the cortex, yet leave it still accessible to afferent impulses. The secondary discharge thus presents interesting problems. What is its essential nature? What structures are involved, and how is activity distributed among them? To throw some light on these questions we performed a series of experiments of which this is a preliminary report.

METHOD

Cats were used in all experiments In most of them pentobarbital sodium (nembutal) was the only anesthetic In some cases dial was used, and in some ether was superposed on the pre existing pentobarbital anesthesia. Our procedure falls in two main groups examination of the distribution of the electrical effects in different parts of the cortex, and comparison of the latencies of their various components with those which could be measured in the afferent pathways leading to the cerebral cortex Sometimes both sciatic nerves were stimulating in the course of a single experiment, sometimes only one In all cases stimulating electrodes like those used by Derbyshire, Rempel, Forbes and Lambert (1936) were secured in contact with the nerve throughout the experiment For stimuli, single induction shocks were delivered from a Harvard inductorium by means of a hand-operated mercury-contact key The strength of stimulation was maximal or nearly so for the flexion reflex * Time of stimulation was recorded on the film by a small string galvanometer throwing its shadow on the margin of the film (see Forbes and Cattell, 1924)

Recording was done with a Hindle string galvanometer and a direct coupled amplifier (Forbes and Grass, 1937) The film was run at a high enough speed to facilitate the measurement of latency After the response had been recorded from the intact cerebral cortex, various extirpations were performed and their effects on the response were observed Often in a single experiment, several successive portions of one or both cerebral hemispheres were removed, and responses were recorded after each extirpation Various types of lead-off electrodes were used In nearly all cases they were of the Ag-AgCl type In some experiments they were in the form of plates of several sq mm in area More often they were of wire presenting a surface of much less than a square millimeter Often two such wires were cemented together as a "bipolar" pair In one significant experiment one wire of each pair projected about 2 mm beyond the other and could thus be inserted into a layer of active cells while its mate rested on the surface of the cortex A similar method of leading off was sometimes achieved with a pair of concentric electrodes, as described by Beecher, McDonough and Forbes (1938) In a few experiments a metal plug was screwed into the cranium and through a hole drilled in the plug a needle was inserted until it touched the dura

In several experiments comparisons were made of different types and arrangements of electrodes for leading off the cortical potentials Three arrangements were directly compared in a number of records in each of three successive experiments These three were as follows (i) Plates applied, as in the experiments of Derbyshire, Rempel, Forbes, and Lambert (1936), one to the dura over the intact cortex, the other (ground lead) to a cauterized area on the opposite cortex (ii) A plug screwed into the cranium was the ground lead, the needle (grid lead) penetrated the dura and about 0 5 mm into cortical gray matter (iii) The needle which passed through the plug, as grid lead, the plate on the cauterized area, as ground In all three experiments lead ii (needle-plug) regularly gave the largest excursions in the recording system, its voltages amounting often to over 500 μV , or about double the average voltage obtained with either of the other two arrangements (Fig 1) In one of these experiments the gain in the amplifier was adjusted to compensate for this difference, the resulting comparison showed no difference in wave-form between the two first named methods of recording

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* Observation of the action current in the sciatic nerve with a cathode ray oscillograph indicated that the stimuli used were well above maximal for A fibers, about threshold for B fibers and probably were usually below threshold for C fibers.
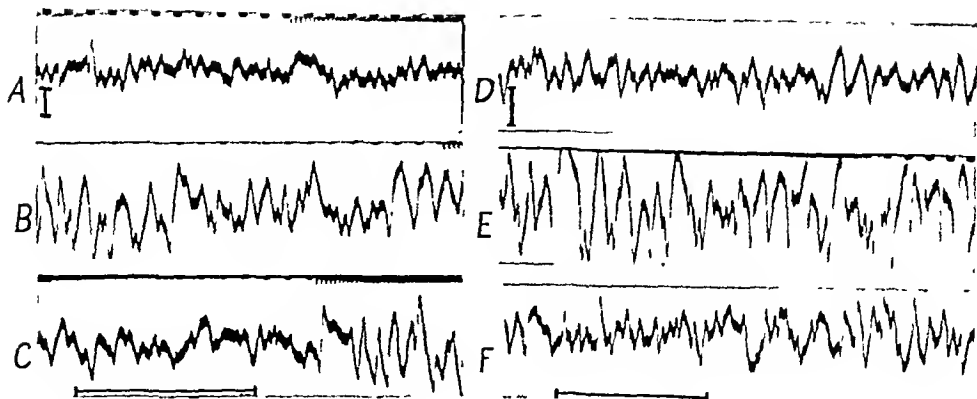
FIG. 1. Activity from cat's sensorimotor cortex under moderate pentobarbital-sodium anesthesia, recorded with different electrode combinations. A, B, C, one experiment; D, E, F, another. A, D: two plates, grid on active cortex, ground on cauterized area. B, E: concentric electrodes with wire to grid, plug (on skull) to ground. C, F: wire to grid, plate on cauterized area to ground. *String galvanometer.* One sec. shown by horizontal lines underneath records. Vertical line beside records represents 200 μV. In each series the amplification was kept constant throughout. In this and in all subsequent records the upward deflection of the string signifies grid negative.

FIG. 2. Responses to sciatic-nerve stimulation from sensorimotor cortex under deep pentobarbital sodium anesthesia, showing both primary and secondary discharges. Diagram shows position of electrodes in these three cases, indicating distribution of positive and negative potentials. Bipolar electrodes. Anterior electrode connected to grid. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 100 μV. Time of stimulation (single shocks) shown by arrow and also by small initial excursions due to electric artifact.

In several experiments we applied a pair of ' bipolar" electrodes (fine silver wire, 0 5 to 2 mm apart), resting on the cortex Although, in most cases, the spontaneous potentials and secondary discharge thus recorded were much smaller than with the other methods in some experiments they were as large as with the other types of electrodes (500 μV or more) This is somewhat surprising, since it suggests a steep potential gradient between adjacent points in areas of the cortex which might be expected to be affected in like manner by afferent volleys and therefore to remain nearly equipotential with respect to one another It was feared that the apparatus might not be recording a true difference of po tential between the leads on the cortex, but rather a transient difference of potential be tween the grid electrode and the ground, in which the capacity to earth of the animal served as the return lead This was controlled by reversing the lead wires without disturb ing the contacts of the bipolar pair of electrodes with the brain The resulting reversal of excursions in the galvanometer showed that we were indeed recording a difference of po tential between nearby points on the cortex These bipolar leads were also used to explore
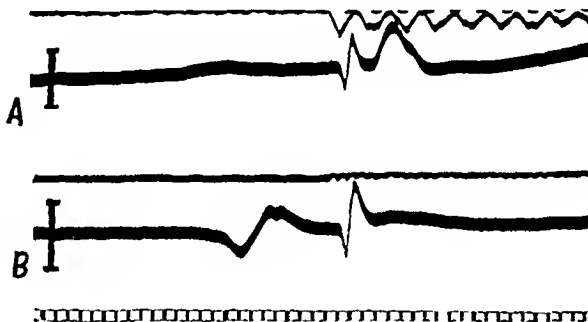
Fig 3 Effect of recent spontaneous activity on secondary discharge under deep pentobarbital sodium anesthesia Primary response unaffected Electrodes grid, wick, on sensorimotor cortex, ground, plate on skull In B spontaneous wave precludes secondary discharge Time (0 01 sec) shown below records Vertical line beside records represents 500 μV Time of stimulation (single shocks) shown by initial excursion of signal line on top

the various parts of the cortex In one experiment there was a systematic change in the polarity of the secondary discharge as the leads passed a certain critical point This is illustrated in Fig 2, in which the accompanying diagram shows where an area became positive during the discharge with respect to adjacent areas both behind and in front of it We have not explored the cortex enough to make further statement about this distribu tion * It seemed probable that a more significant arrangement of leads was from interior to surface a view supported by the larger voltages obtained when the grid lead penetrated the gray matter (see above) The concentric electrodes described by Beecher, McDonough and Forbes (1938), in which the grid lead penetrates about 2 mm into the cortex, are perhaps the most satisfactory

RESULTS

Forbes, Renshaw and Rempel (1937) described isolated spontaneous discharges of the cortex in the deeper stages of pentobarbital narcosis In

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* It is of interest to note that in A and C of Fig 2 the spontaneous waves in each case are of opposite sign to the secondary discharge
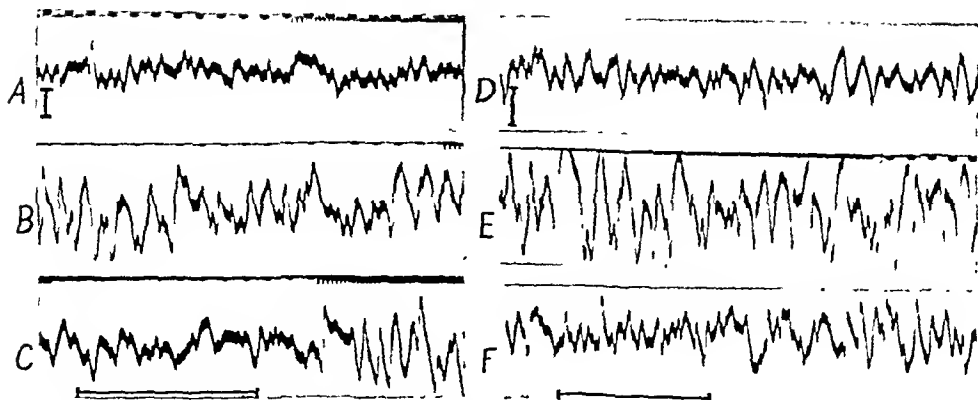
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mary response, on the other hand, although far less susceptible to fatigue or extinction by previous activity, changes far more than the secondary discharge as the electrodes are moved to different locations. It generally decreases rapidly or completely disappears as soon as the electrodes are more than 6 to 8 mm. away from the point of maximum activity in the sensorimotor area. Figure 5 illustrates this point. This difference is also illustrated in those experiments in which ipsilateral and contralateral responses were
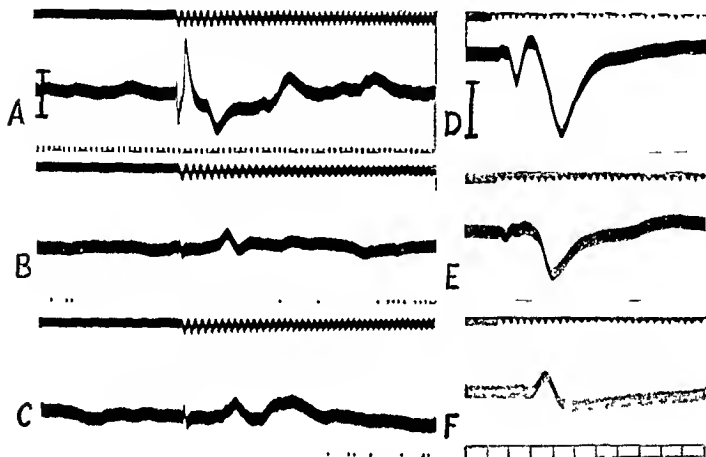
Fig 5 Responses to sciatic-nerve stimulation from different parts of the cortex under deep pentobarbital sodium anesthesia, showing dependence of primary response on proximity to sensorimotor cortex A, B, C, one experiment, D, E, F, another A, D sensorimotor cortex B, E anterior marginal gyrus C, F: posterior marginal gyrus *Time* in A, B, C, 0 01 sec , in D, E, F, 0 04 sec , shown below records Vertical line beside records represents 200 μV Line on top of records, signal magnet

compared, either by stimulating alternately right and left sciatic nerves or by recording alternately from corresponding points on right and left hemispheres. They regularly showed greater differences between the primary responses of the two sides than between their secondary discharges. This difference in the majority of our experiments consisted in the complete absence of the primary response on ipsilateral stimulation (Fig. 6). In some experiments this response was discernible but of opposite sign and much smaller than the response to contralateral stimulation. The latency of the ipsilateral response then was usually the same as that of the peak of the primary response on the contralateral side. But following the primary response was a secondary discharge of approximately the same latency and

usually of the same wave-form and magnitude, whichever nerve was stimulated or from whichever cortex it was recorded.

In a few experiments, to a pre-existing deep pentobarbital anesthesia, in which good primary and secondary responses were elicited, ether was added. All these experiments showed no change in the primary response, but a striking increase in the latency of the secondary discharge from about 50 msec. to 150 msec. in about 15 min. The voltage decreased from 150 μV. to 60 μV., after which only the primary response was present. After the ether was taken off, the secondary discharge reappeared with a latency of 150 to 160 msec. and of small voltage. In about 6 to 10 minutes, the voltage and latency returned to their original values (Fig. 7). When ether was added again and continued until death, the primary response persisted long after the second-
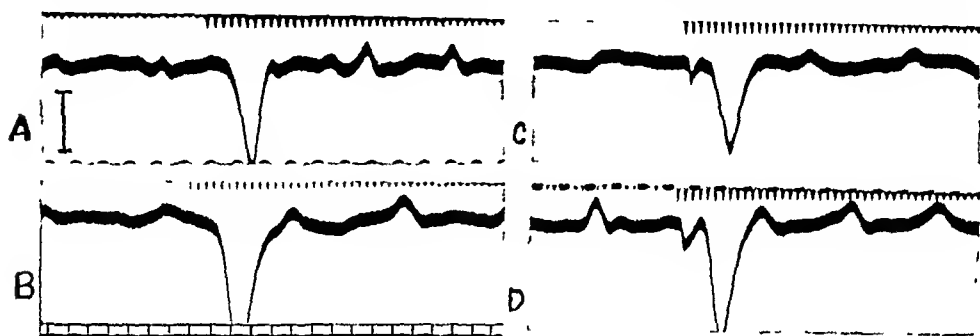

Fig. 6. Differences in responses of sensorimotor cortex to ipsilateral and contralateral stimulation under deep pentobarbital sodium anesthesia. Left column, ipsilateral stimulation; right column, contralateral stimulation. A, C: left cortex; left and right sciatic-nerve stimulation. B, D: left and right cortex; left sciatic-nerve stimulation. *Time* (0.04 sec.) shown below records. Vertical line beside records represents 200 μV. Time of stimulation (single shocks) shown by signal magnet on top of each record.

ary discharge had disappeared; it was abolished just at exitus. Usually great increase in depth of pentobarbital anesthesia caused only a slight increase in latency, although in one exceptional experiment increased pentobarbital narcosis resulted in almost as great an increase of latency as when ether was added (cf. Marshall, 1938).

It has already been emphasized (cf. Derbyshire, Rempel, Forbes and Lambert, 1936, p. 588) that only when the cortex is rendered quiescent by deep barbiturate or similar narcosis do the primary and secondary responses herein described become evident. Some of our experiments show an actual increase in the size of the primary response upon increasing the depth of narcosis, and indeed an even greater relative increase than the corresponding augmentation of the secondary discharge. In one experiment with the needle-plug electrodes, the needle electrode was thrust progressively farther into the gray matter and then through it into the underlying white matter, the ground lead remaining fixed in the cranium throughout the series. The primary response changed little in this series, whereas the secondary discharge

showed a great change in size, form, and phase relations, illustrated in Fig. 8.

From the above observations on latency and localization, it may be inferred that the primary response is associated with afferent impulses in the sensory paths approaching the cortex. The great decline in this response as the electrodes are moved a few millimeters away from the sensory area denotes a fairly sharp localization of this effect in that area. The secondary discharge, on the other hand, appearing about 30 or 40 msec. after the onset of the primary response, occurring in widely separated areas in the cortex, and presenting a similar waveform in corresponding points on the two hemispheres simultaneously, denotes a widespread cortical disturbance which is presumably evoked by the afferent volley. Further evidence concerning the structures involved in these two dissimilar events was sought in a number of extirpation experiments.

In some experiments we first recorded the response to sciatic-nerve stimulation from the cortex and then removed portions of the cerebrum, exposing the hippocampus or decerebrating the cat. The responses recorded from the afferent path near the anterior end of the hippocampus or from the brainstem corresponded in their time relations closely with the primary responses from the cortex. This primary response, whether recorded from the cortex or from the afferent
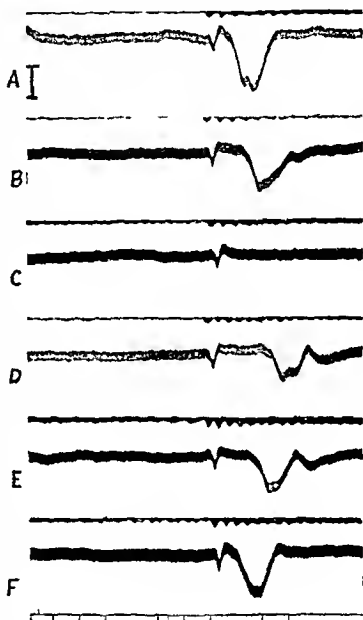

FIG 7. Effect of ether added to deep pentobarbital sodium anesthesia on the response to sciatic-nerve stimulation A· deep pentobarbital sodium anesthesia B: 10 min. after beginning of etherization C: 8 min. after B D: 2 min after withdrawal of ether. E: 1 min. after D. F: 9 min after E *Time* (0.01 sec.) shown below records Vertical line beside records represents 100 μV. Time of stimulation (single shocks) shown by signal on top of each record.

path, was sometimes simple, and sometimes dicrotic, as in the corresponding records published by Forbes and Miller (1922). The identification of these two peaks with those appearing in the records of Forbes and Miller is substantiated by the differential effect on them of both ether and repeated stimulation, the second peak always decreasing the more.

The dependence of the secondary discharge on the cerebrum was shown by the following experiment, illustrated in Fig. 9 (ground lead on skull throughout). After recording the responses from both cortices to stimulation of one sciatic nerve, unilateral decerebration was performed, the hemisphere on the same side as the stimulated nerve being removed. The electrode which
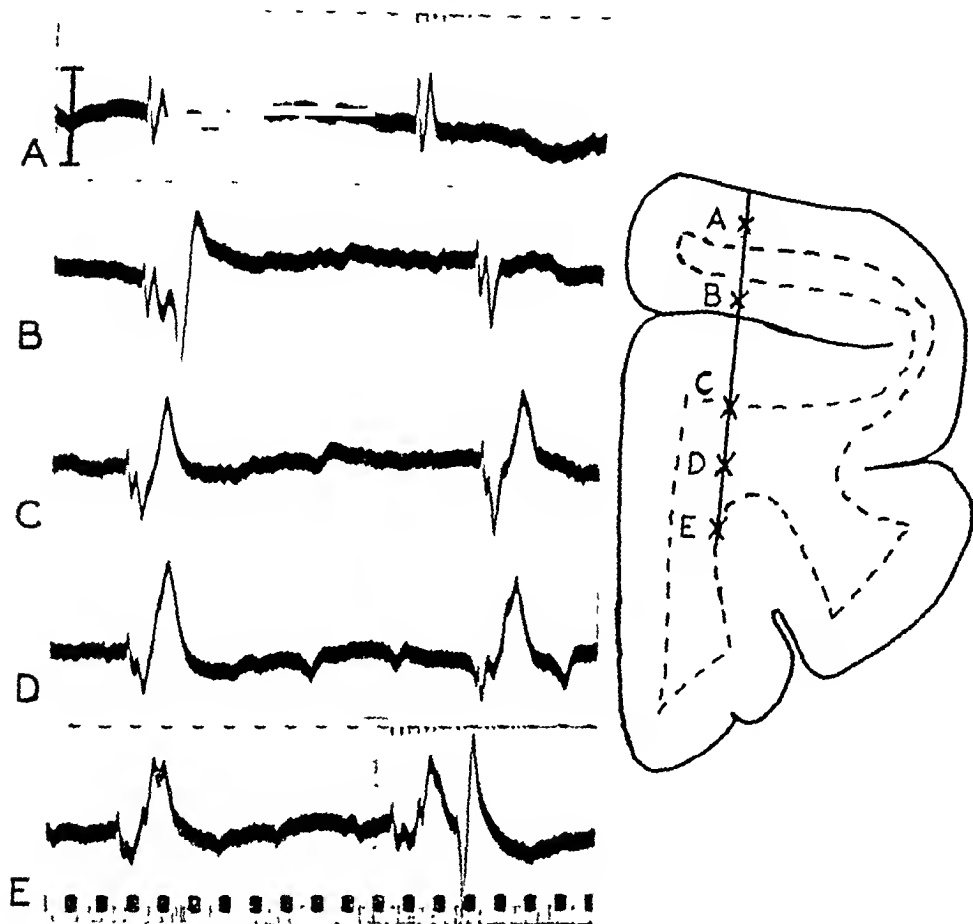

FIG 8. Changes in the secondary discharge when one electrode penetrates progressively into the brain. Diagram showing section of brain with track made by the electrode. Crosses show approximate position of grid electrode corresponding to each of the records. Ground lead in skull. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 100 μV. In each record are shown the responses to a consecutive pair of shocks, make and break.

had been on that cortex was moved to the cut brain-stem, while the other grid lead remained on the intact cortex. A few minutes after this operation the secondary discharge, led from the intact cortex, showed a latency about three times as long as before decerebration. This latency decreased gradually until at about 10 min. after decerebration it had returned to its original

value. The size and shape then were similar to those recorded before decerebration. When records were taken from the lead on the cut brain-stem the secondary discharge was recorded, greatly reduced in size (Fig. 9B), as would be expected in the case of active tissue remote from the electrode. Decerebration of the second side abolished the secondary discharge on both sides of the brain-stem. A well-defined primary response was then recorded from the side contralateral to the stimulated sciatic nerve. On the ipsilateral
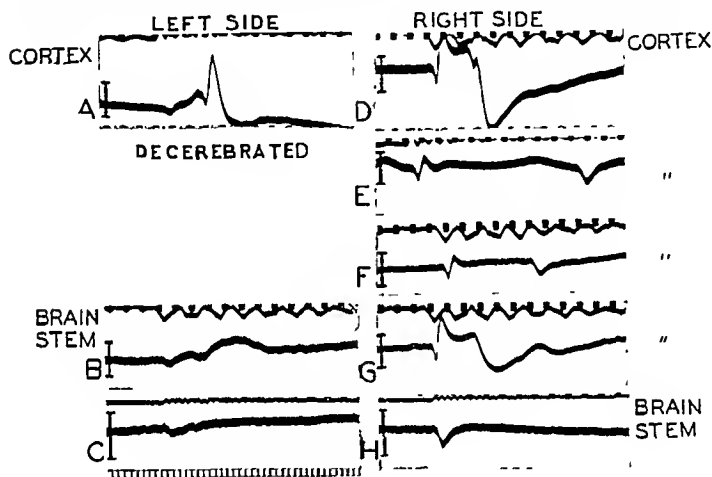

FIG. 9. Effect of unilateral and bilateral decerebration on the responses to sciatic-nerve stimulation. Electrodes: wicks on left and right cortices, grids; plate on skull in midline, ground. A and D: intact cortex; left sciatic nerve stimulated. After A and D, left side decerebrated and electrode from left cortex placed on brain-stem. E, 3 min., F, 4 min., G, 10 min., after decerebration. B record from brain-stem, 14 min. after decerebration. Right side decerebrated. C: left cut surface. H: right cut surface. *Time* (0.01 sec.) shown below records. Vertical line beside records represents 200 μV. Line on top of records, signal.

side only a small primary response was recorded. The large but transient increase in the latency of the secondary discharge after hemidecerebration may have been a consequence of the anemia caused by ligating the carotid arteries and compressing the vertebrals in order to prevent hemorrhage. Beecher, Forbes and McDonough (1938) found that lowering of blood pressure produced changes closely resembling those of increased depth of anesthesia (cf. Adrian and Matthews, 1934, p. 468), and we have mentioned above that these changes sometimes include greatly prolonged latency of the secondary discharge.

In other experiments designed to localize the secondary discharge more

closely in the cortex, several pairs of bipolar electrodes were placed on different parts of the cortex. The usual arrangement was as follows: The first pair of electrodes was placed 2 to 3 mm. posterior to the cruciate sulcus and about 5 mm. from the midline; the second behind the sulcus ansatus on the anterior third of the marginal gyrus; and the third on the marginal gyrus about 10 to 15 mm. posterior to the second. After responses to sciatic-nerve stimulation had been recorded from all three positions, an incision was made
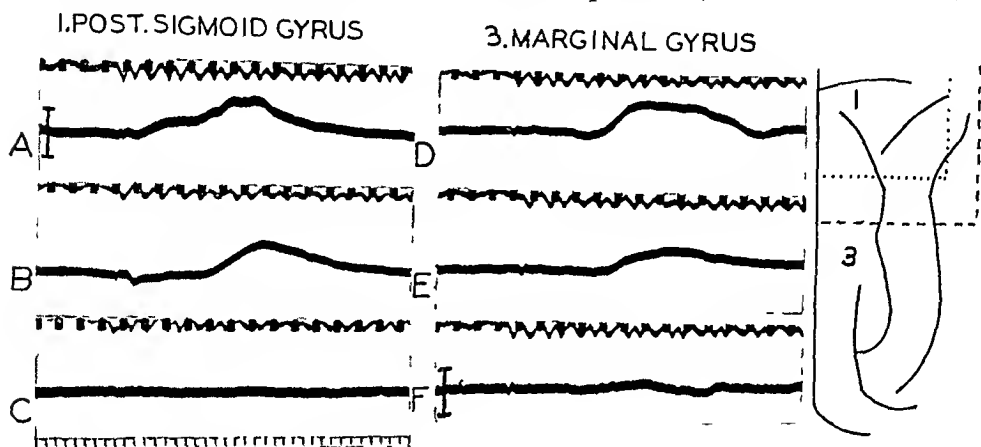

FIG. 10. Effect of cortical extirpations on the secondary discharge under deep pentobarbital anesthesia. Diagram shows positions of the electrodes. A and D, intact cortex. Before B and E, part of cortex anterior to dotted line was extirpated. B, on cut surface beneath position 1. E, still in the same position. Between E and F, corresponding part excised from left cortex. C, after the part of cortex indicated by broken line was extirptaed on both hemispheres. A, B, C, D, E, same amplification. Vertical line beside records represents 200 μV. F, higher amplification. Vertical line in F, 100 μV. *Time* (0.01 sec.) shown below records. Line on top of records, signal.

between electrodes 1 and 2. The responses recorded after that from all three leads were usually decreased in size, but after a lapse of time attained nearly their previous size. A second incision between electrodes 2 and 3 had the same effect. Removal of the cortex anterior to the first incision decreased the responses from the cut surface to which electrode (or electrodes) 1 was reapplied, and even more so from the posterior electrodes which were still in place. Small secondary discharges on the operated side, and larger ones on the intact side, continued to be recorded until the frontal part of the cortex as far as the anterior third (approximately) of the marginal gyrus on both sides had been removed. With this extirpation the secondary discharge was completely abolished. Such an experiment is illustrated in Fig. 10.

DISCUSSION

The experiments of Marshall, Woolsey and Bard (1937; see also Bard, 1938) with sharply localized tactile stimuli, in both cat and monkey, show correspondingly localized cortical potentials. Their latencies and durations are nearly the same as those of the primary response in our experiments. On repeated stimulation the responses decrease in size and disappear at fre-

quencies of from 12 to 15 per sec. We have not used such high frequencies as this, but, since with frequencies up to 7 per sec. the primary response continues to appear with more than half its initial size, it seems unlikely that at double that frequency our primary responses would decline to extinction. Probably the conditions of our experiment—*viz.*, strong stimulation of the entire sciatic nerve—are more favorable to persistent response. Possibly the response which Bard and his co-workers have recorded is from a mechanism intermediate between those whose activity is patent in the primary response and secondary discharge in our records, and is more localized than our widespread secondary discharge.

The time of the primary response has been mentioned loosely as showing the time of "arrival in the cerebrum" of the afferent volley. It is desirable to be more specific, if possible. Leese and Einarson (1934) showed that the first response in the medulla oblongata, when the sciatic nerve was stimulated as in these experiments, appeared about 6 msec. after the stimulus. Forbes and Miller (1922), recording from the cut brain-stem at the colliculi, found after the medulla response a second excursion whose peak was attained at about 20 msec. after the stimulus. We find that whether we lead from the sensorimotor cortex or from the afferent path near the anterior end of the hippocampus, the first excursion begins about 10 msec. and reaches its peak about 20 to 22 msec. after the stimulus.* In those preparations which we decerebrated after recording from the cortex, the major excursion recorded from the brain-stem showed approximately these same time relations. The conclusion seems to be that conduction from the level of the colliculi to the sensory cortex is rapid. The peak of the wave recorded from the sensory area probably represents the actual arrival of the afferent impulses, and we may infer that they reach the cortex not over 25 msec. after the stimulus is applied to the sciatic nerve. Moderate slowing of the primary conduction time has sometimes been found under deep barbiturate narcosis. Much more marked is the slowing of the secondary discharge under like conditions. In general the latency of the latter is much more variable than that of the primary response. The extreme example of this is the increase of latency to 150 msec. when ether is superimposed on barbiturate narcosis.

Probably the most significant feature of the secondary discharge is its complete disappearance when the stimuli are applied with frequencies greater than 3 or 4 per sec. This effect suggests a compound rather than a simple system. If a nerve or muscle is stimulated with a frequency higher than it can follow, it will respond rhythmically at a frequency determined by its refractory period. This is most strikingly shown by the rhythmic contractions of the heart in Stannius ligature when a rapid succession of stimuli is applied. The behavior of the secondary discharge in the cortex is entirely different. A rapid series of stimuli evokes only one response; as long as the stimuli continue there is no recurrence.

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* In the most clearly defined records of this type the localizing electrode in the sensory path became negative.

Perhaps the simplest way to explain the disappearance of the secondary discharge on rapidly repeated stimuli would be to suppose that it is evoked through some mechanism whose threshold is such that only after a rest is the afferent volley (primary response) strong enough to be an adequate stimulus, and that with the demonstrable decline in the primary response to a depressed level it falls below the threshold. To this explanation, it might be objected that the afferent volley when it reaches the cortex consists of impulses in axons responding on the all-or-none principle, whose relative refractory periods are much less than 0.25 sec. This may be answered by the probability that the *number* of converging impulses determines the strength of the volley as a stimulus to the cortical synapses. Indeed the obvious fact that the primary response declines when repeated more than 5 times a second shows that the available energy is in some way reduced. A more serious objection to the simple explanation proposed is that the observed decline in the primary response is relatively small—often not more than 20 or 25 per cent. With so small a difference in strength between the first and subsequent volleys, one would expect the extinction of the secondary discharge after all but the first volley of a series to occur only in a narrow range of depths of narcosis, for soon after the excitability had decreased enough to render the later volleys ineffective its further decrease would render even the first (full-sized) volley ineffective. But this is not the case. The secondary discharge is found over a wide range of depths of narcosis, and as far as our observations have gone they seem to show that whenever it can be evoked it can also be made to disappear on repetition of stimuli.

As intimated in a previous paper, the effect may perhaps be the result of inhibition. If so, the inhibitory effects of the afferent volleys must continue to dominate the discharge as long as these are delivered above the requisite frequency of 4 or 5 per sec.

The great mass of data which we have gathered concerning the "alpha rhythm" of Berger suggests the existence of a "pace-making" mechanism which tends to synchronize the discharge of great quantities of cortical cells. The evidence of Derbyshire, Rempel, Forbes, and Lambert (1936) that pentobarbital in its early stages increases the amplitude of the major spontaneous waves without disturbing their rhythm suggests that the tendency of large groups of cells to synchronize is increased by moderate doses. As the narcosis deepens, the waves become more infrequent but show little change in their individual time relations. Forbes, Renshaw and Rempel (1937) described deep stages of pentobarbital narcosis in which isolated waves, individually of similar time relations to those in the lighter stages, rise from a smooth base line, sometimes at regular intervals, but in the deeper stages at long and irregular intervals. In the deepest stages these spontaneous waves disappear and the base line is continuously smooth.

The secondary discharge can be evoked at any stage of narcosis which offers a smooth base line even for a fraction of a second, but, as already noted, the spontaneous waves prevent the discharge from appearing if the

stimulus is applied too soon after their cessation. This strongly suggests that the same pace-making mechanism which synchronizes the spontaneous discharge of the cells may also initiate the widespread disturbance which constitutes the secondary discharge. This is further suggested by the fact that the spontaneous waves, though sometimes dissimilar, often closely resemble the secondary discharge in direction, duration and wave-form. The pace-making mechanism might be pictured as normally active and initiating waves of more or less regular rhythm, and becoming progressively less prone to discharge as narcosis deepens, although the cells they synchronize are still able to respond. The pace-maker would then discharge less frequently until finally it became quiescent. But even then it could be stimulated by an afferent volley and thus made to set off the secondary discharge. If we further suppose that the pace-maker is subject to rapid fatigue, we can explain its failure to set up more than one secondary discharge when afferent volleys arrive at excessive frequencies. But it will not suffice to ascribe the effect simply to a long refractory period in the pace-maker, for in that case it would recover and initiate discharges later in the series of stimuli. Two explanations of its continued failure throughout the series occur to us. We may suppose either that the refractory phase of the pace-maker, set up by the first volley, is maintained by some effect analogous to the post-cathodal depression described by Erlanger and Blair (1931), or that the pace-maker is excited by each volley to some degree of activity, but that narcosis and fatigue conspire to make its response too small to initiate the widespread discharge.

Bishop and O'Leary (1936) showed that in the lightly etherized rabbit, stimuli applied to the optic nerve evoke in the optic cortex surface-positive waves of somewhat similar time relations to the secondary discharge which we have observed. They ascribe them to corticifugal impulses travelling to the thalamus. More recently three sequences of potentials have been identified in the optic cortex (Bartley, O'Leary and Bishop, 1937, rabbit; Bishop and O'Leary, 1938, cat). Application of strychnine has furnished reasons for associating these sequences with different neural mechanisms. In both animals the slowest waves (ascribed to corticifugal impulses) are taken to involve the same mechanism as the alpha waves. In our experiments the long duration of the secondary discharge, its wide distribution and its apparent involvement of the same mechanism as spontaneous discharges resembling alpha waves, all suggest that it is of the same kind as the slowest waves described by Bishop and his co-workers. Like these it may well be a corticifugal discharge, and in favor of this view is the fact that when recorded with a large surface electrode referred to a ground lead on remote inactive tissue, the secondary discharge is regularly surface positive in all regions of the cortex close to the sensorimotor area.

Adrian (1936), stimulating the rabbit's cortex directly with brief electric currents, has reported a spread of surface-positive waves, referable to activity of cells in the deeper layers of the cortex, radiating from the stimulated point. If we look on the secondary discharge as a similar spread from the

sensory area, we encounter two difficulties. The discharge does not appear surface-positive in all parts of the cortex, but presents differences in shape and sometimes in polarity in different areas (cf. Derbyshire, Rempel, Forbes and Lambert, 1936). More difficult is the fact that, although there is a lag of 20 to 50 msec. between the primary response and the secondary discharge at the sensory area, the secondary latency usually increases so little as the leads are placed farther away from the sensory area as to indicate conduction velocities of 50 to 100 cm. per sec., which exceed the range of velocities (10 to 40 cm.) observed by Adrian. These facts suggest that the disturbance spreads over the cortex via some more rapidly conducting channels than those chains of neurons which were involved in propagating the waves described by Adrian, or that the velocities in corresponding chains are more rapid in the cat than in the rabbit. A third alternative is that the afferent paths deliver the incoming volley not only to the sensory cortex, but also the thalamus or other subcortical centers, and that from there impulses are distributed widely in the cerebral cortex. This explanation would readily account for the delay of 20 msec. or more after the arrival of the afferent volley before the appearance of the secondary discharge in the sensory cortex. It would also explain the brief and variable interval between the arrival of the secondary discharge at the sensory area and at more remote regions of the cortex, for the subcortical centers can easily be supposed to deliver this disturbance in many parts of the cortex almost simultaneously. Incisions in the cortex between the sensory and other areas have furnished evidence both for and against this view. In some cases they have resulted in a great decrease in the discharge recorded from the regions thus cut away from the sensory area. In other cases the discharge has persisted with little change after such incisions have been made. There is need for further research on this particular problem.

Bremer (1937) has argued that barbiturates act selectively on internuncial neurons in the cortex, rather than on afferent or motor paths. The secondary discharge, representing cortical activity which finds no motor expression in the skeletal musculature, would seem to be most easily explained as a discharge of internuncial neurons, and is therefore hard to reconcile with Bremer's view. But the structure of the cerebrum is so complex and includes so many different kinds of neuron that one can conceive of one type being narcotized while another type remains excitable. It is not certain, therefore, that our evidence cannot be reconciled with Bremer's view.

Perhaps the quiescent state of the cortex under deep barbiturate narcosis, even when it is clearly capable of activity in response to sciatic stimulation, may be explained by a rise in threshold in its channels of approach such that only massive stimulation can break through. Possibly, as Beecher, Forbes and McDonough (1938) have suggested, the ordinary streams of sensory impulses fail to excite the cortex because of its high threshold, yet the mass of convergent impulses due to maximal stimulation of the sciatic nerve produce a central effect adequate to attain the threshold and evoke a generalized discharge.

SUMMARY

1. A previous paper described a widespread electric response of the cerebral cortex to stimulation of the sciatic nerve appearing in the deeper stages of narcosis with pentobarbital or avertin. We propose to call it the "secondary discharge." The same effect is found with dial (di-allyl barbituric acid).

2. The secondary discharge is invariably characterized by its immediate and complete extinction when the initial stimulus which evokes it is followed by succeeding stimuli at frequencies above about 3 per second, by the need of a rest of considerably more than a second before a full-sized discharge can again be evoked, by its subnormal magnitude if evoked at intervals between about 0.5 sec. and at least 1.5 sec., and by its complete failure to reappear after its initial extinction even if the rapid series of stimuli is continued for several seconds. In like manner, shortly after a spontaneous wave the secondary discharge cannot be evoked. In these respects it is sharply differentiated from the primary response which precedes it, whose magnitude declines only slightly in the successive responses to rapidly repeated stimuli.

3. The secondary discharge is widespread in the cerebral cortex, appearing with nearly the same latency and duration in both hemispheres in response to stimulation of one sciatic nerve, and with similar time relations (though sometimes with reversed polarity) in regions of the cortex remote from the sensory area.

4. On stimulation of one sciatic nerve, the primary response differs far more when compared in the two hemispheres than does the secondary discharge which follows it. A few millimeters away from the sensory area, the primary response may not be discernible, although in the same preparation it exceeds the secondary discharge in voltage when recorded from the sensory area for the sciatic nerve (Fig. 5).

5. The latency of the primary response in the sensory cortex identifies it with the afferent volley, which can be recorded at various points in the afferent path from the medulla to the cerebral cortex.

6. Incisions through the cortical gray matter between the sensory area and other regions to which electrodes were applied, in some cases reduced the response in a manner that suggested a spread of the disturbance from the sensory area by way of the cortical gray matter. But the long delay between the primary response and the secondary discharge, and the approximate synchronism of appearance of the discharge in widely separated regions of the cortex, suggest that the afferent volley may act upon the thalamus or other subcortical centers, and that these centers may then distribute the discharge throughout the cortex.

7. The failure of all but the first of a series of repeated stimuli above a critical frequency to evoke the discharge suggests the existence of an intermediate mechanism between the afferent tract and the cortical cells whose discharge is recorded. A pace-making mechanism, normally producing the synchronized rhythmic discharge of cortical cells, having its threshold raised by the narcotic and in this state rapidly fatigued, might be the intermediate

mechanism whose failure would explain the extinction of the response to repeated stimuli.

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A SEARCH FOR CHANGES IN DIRECT-CURRENT POTENTIALS OF THE HEAD DURING SLEEP

H DAVIS, P. A DAVIS, A L LOOMIS, E N. HARVEY, AND G. HOBART

From the Department of Physiology of the Harvard Medical School, Boston, and The Loomis Laboratory, Tuxedo, N Y.

INTRODUCTION

DURING SLEEP the pattern of electrical activity recorded from the human head differs widely from the waking pattern (Loomis, Harvey, and Hobart, 1937, 1938). As sleep becomes deeper the electrical waves increase in voltage and become longer and longer until the time from crest to crest becomes as great as 4, 6, and even 10 sec. Such slow changes of potential tax the ability of available capacity-coupled amplifiers (time constant = 0.5 sec.) and suggest the possible existence of still slower changes, perhaps even changes in steady state directly related to fluctuations in the depth of sleep. Slow changes in the potentials of the brain (so-called "D.C. changes") correlated with changes in physiological state are also suggested by the observations of Cohn and Langenstrass (1938) on patients in insulin coma and by Burge, Wickwire, *et al.* (1936) on animals under various depths of anesthesia. Another instance of systematic change in D.C. potential is the "ovulation potential" (Burr, Hill, and Allen, 1935; Reboul, Davis, and Friedgood, 1937). The transient "galvanic skin reflex" is a familiar phenomenon. Because of these various suggestions of the existence of significant D.C. changes correlated with changes in physiological state we undertook to observe the changes in D.C. potentials on the human head during sleep.

METHOD

Apparatus Two fundamentally different methods of amplifying and measuring D.C. potentials have been employed The first is a push-pull direct current amplifier and mirror galvanometer The scale is conveniently read to 0 5 mm , corresponding to 0 05 mV of potential-difference between the input leads The amplifier is stable and convenient in operation The input is balanced and a large cathode resistor is used (Fig 1), consequently the instrument is quite insensitive to movements of the subject and to all unwanted (in phase) potentials It is unnecessary to ground the subject or to take any special precautions to insulate him from ground Stability of the amplifier is improved by never turning the set off, as the cooling and reheating of the tubes is liable to change the balance of the set A trickle charger keeps the filament battery charged

The other method depends upon converting a steady potential-difference into a pulsating current by means of a mechanical interrupter The brief pulses are accurately amplified by condenser-coupled amplifiers and recorded simultaneously with the usual electroencephalogram by means of a multi channel ink-writer (Loomis, Harvey, and Hobart, 1938) To use this latter method on our regular 6-channel oscillograph it was only necessary to connect a motor-driven short-circuiting switch across the input of each circuit desired to use as a D C circuit The input of each amplifier was thus short-circuited 5 times a sec. When the short-circuiting switch was closed, there was no voltage between the grids of the first pair of tubes When the short-circuiting switch opened, the D C potential which we wished to measure appeared between the input leads, and the recording pen made a corresponding excursion The pen returned to its base-line when the switch closed again

Carefully prepared silver contacts proved adequate to avoid trouble from contact potentials when the short circuit was closed. The sensitivity of the amplifiers was adjusted so that when there was 1 mV. across the input the amplitude of the square waves produced by the opening and closing of the short-circuiting switch was 1 cm. Thus all D.C. voltages of less than 1 mV. could be read directly from the record. For larger voltages a compensating voltage to the nearest even millivolt was placed in series in the input circuit and the number of millivolts thus added was automatically recorded on the record. The great advantage of this method for long runs is that the calibration is dependent only on the compensating voltage and is not dependent in any way on the "drifting" of the amplifiers.

Electrodes. Electrodes present a special problem in studies of D.C. potentials. In preliminary experiments silver-silver chloride half-cells were employed resembling those de-
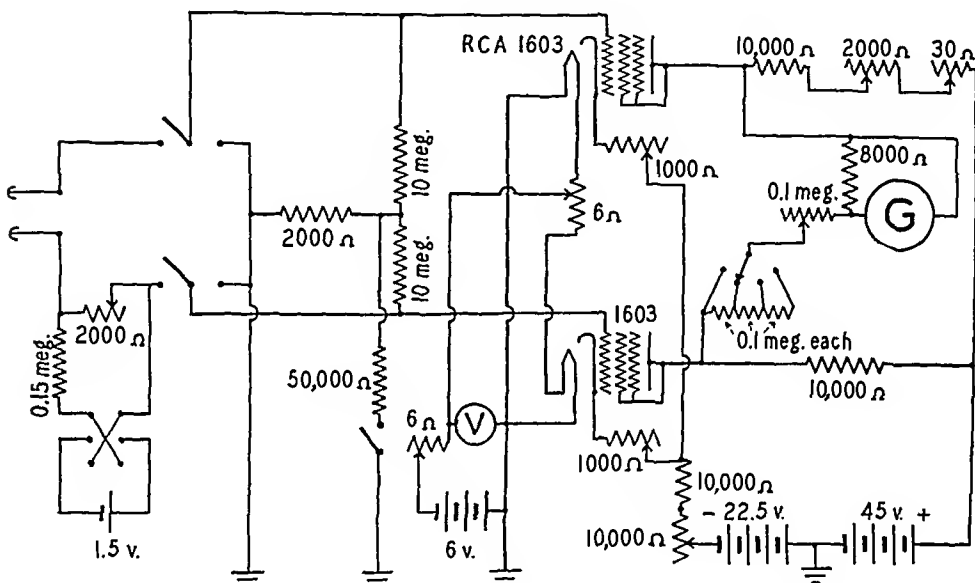
VACUUM TUBE VOLTMETER

Fig. 1. Wiring diagram of balanced-input D.C. millivoltmeter.

scribed by Burr, Lane, and Nims (1936). Such electrodes are stable and equipotential far beyond the requirements of the present type of experiment, but are difficult to attach to the subject without leakage from the salt bridges. They are excellent for brief explorations, but troublesome for a night's sleep. Miniature Ag–AgCl–NaCl electrodes were devised, each one less than 1 cm. in height and diameter. These were small cup-shaped cells which were molded from transparent methyl methacrylate in such a way that there was a long spiral capillary tube in the wall of the cell, opening to the exterior. The capillary allowed for contraction and expansion of the fluid within, without escape of fluid or admission of air. These cells were satisfactory in their electrical stability and for short runs, but it was difficult to keep them on a subject's head when he tossed during sleep. Two fairly simple and mechanically convenient combinations with adequate electrical stability were evolved, one employing zinc, the other copper, as the metal.

From sheet copper, 0.125 mm. thick, spoon-shaped pieces about 20 mm. long are cut. The "handles" are 1.5 mm. wide, the bowls are circular, 6 mm. in diameter. Enamel-insulated copper wire (No. 32) is attached to the handle by bending the sheet copper over the bared end of the wire and squeezing. This joint involves no other metal than copper, but it is desirable to wrap joint and handle with "scotch tape" (cellophane and an adhesive) to restrict the area of electrical contact to the disc. The copper is cleaned with acid, and just before application is cleaned again with emery paper. The electrolyte is copper

sulphate (4 per cent) in agar (1 5 per cent) jelly Two drops of glycerine per cc are added
to the jelly to delay drying Bits of filter paper slightly larger than the discs are impreg
nated with the jelly and laid on the skin, which has previously been cleaned with acetone
or ether The electrode is attached to the skin by a layer of collodion which completely
covers the electrode, both disc and handle The mechanical attachment is satisfactory,
particularly on the scalp where a few hairs can be drawn across the copper strip for re
enforcement It is not necessary to cut any hairs if jelly is applied liberally beneath the
filter paper The metal strip can easily be bent to conform to the shape of the head Such
copper strips immersed in the $CuSO_4$ agar jelly in a petri dish rarely show potential
differences of as much as 1 mV after the first minute The differences which do appear are
usually stable within 0 2 mV over several hours They show only very slight back E M F
after the passage of a D C current of 0 5 mA The chief disadvantage of this type of elec
trode has been slow drying of the jelly

The zinc electrodes are similar in size and general design to the copper electrodes
already described The zinc is lightly amalgamated by dipping in a dilute $HgCl_2 - HCl$
mixture for a few seconds The solder junction to the copper wire must be carefully pro
tected by insulating lacquer and scotch tape The "bowl" of the spoon is hollowed slightly
to aid in retaining the electrolytic paste A fairly satisfactory paste is Sanborn "Redux "
electrode paste to which has been added 1 gm of $ZnSO_4$ per 25 gm of paste This paste
retains its moisture far better than agar jelly, and is more convenient to keep and to apply
It cannot be used with copper, however, as it reacts with the cupric ion One disadvantage
of zinc is that the zinc electrodes are not perfectly reversible Current flow is not propor
tional to applied voltage for currents of the order of 0 1 mA at 0 01 volts, and there is a
significant back E M F following the passage of such a current The potential differences
among a number of strips dipped in paste may be as much as 3 mV and are a little less
stable than with copper The differences and the variability are small however, compared
with the potential differences and fluctuations introduced by the human skin and in spite
of their obvious imperfections we have employed the Zn-$ZnSO_4$ paste electrodes in nearly
all of the experiments to be described below

In 7 experiments electrodes were placed on the head in the following positions left
and right frontal, 6 cm from the midline at the level of the usual hair line left and right
central 6 cm from the midline in the frontal plane of the auditory meatus, right and left
occipital, 5 cm from the midline at the level 2 cm above the inion, right and left ears"
just behind the ear and above the mastoid process One or two electrodes were also placed
on the upper chest near the midline at the upper end of the sternum

One electrode, usually on the chest, was chosen for reference, and the potential differ
ences between this and each of the other electrodes were measured periodically, usually
every 15 min by means of the vacuum tube millivoltmeter On the 6 channel ink writing
oscillograph, three or four channels were used to record the usual A C brain waves from
various parts of the head The remaining three or two channels were used with motor
driven short circuiting switches to record the D C potentials from three or two other
regions

RESULTS

The electrodes when first placed on the head and chest always showed
potential differences far greater than those found in control comparisons
made in electrolyte paste immediately before applying them Most of the
potential-differences after application were less than 5 mV , but some were
as much as 20 mV The deviations showed no relation to positions of the
electrodes or to the electrical resistances (15,000 to 30,000 ohms) between
them We attribute the deviations to local accidental differences in the con
dition of the skin beneath the electrodes, although the skin was cleaned
thoroughly but gently and care was taken to avoid all cuts or abrasions The
potential differences often shifted gradually by 3 or 4 mV. during the first
half-hour, whether the subject remained awake or went to sleep, and then
either remained quite stable (within less than a millivolt) or attained a slow

rate of drift which might be maintained steadily for hours (Fig. 2 and 3). Neither the initial shifts nor the more prolonged drifts could be related to the position of the electrode or to any other factor which we have been able to identify.

Relation of D.C. potentials to movement. Movements of the subject sometimes caused changes in the D.C. potentials. Often there was a transient shift of 2 or 3 mV. Sometimes the return was slower, requiring from 2 minutes to half an hour (Fig. 3C). All of the abrupt changes in D.C. potential which were observed were associated with movements. There were, however, numerous minor slower fluctuations, of as much as 1 mV. over periods of 1 to 5 min., superimposed on the slower drifts, which could not be related to any apparent cause.

Relation of D.C. potentials to sleep. When the subject fell asleep, as he passed from light to deep sleep, or when he awoke, either gradually or abruptly, no change whatever could be observed in the D.C. potentials of the head. There was no systematic change either between head and chest, between scalp and ears, between frontal and occipital regions or between right and left. Movements occasionally caused their typical abrupt shifts or momentarily obscured the record, particularly on sudden awakening,—but, sleeping or waking, the D.C. potentials between any of the pairs of electrodes continued their slow random drifts. The negative result is consistent in all 7 experiments. We find no change in the D.C. potential-differences or in their "spontaneous" variations which can be related to the state of sleep. In spite of the drifts and the more abrupt alterations resulting from movement, our conclusion can be drawn with confidence for two reasons. First, the large number of electrodes applied to each subject allows us to identify and discount the occasional erratic behavior of an individual electrode and also to average the random drifts of all of them. Occasional D.C. changes at one or two electrodes which occur coincidentally with waking or going to sleep are not reflected at corresponding electrodes on the opposite side of the head and are not reproduced in other experiments. Second, the major changes in potential are either much slower than the changes in the state of sleep, or else (when associated with movement) they are much quicker.

The usual electroencephalogram reveals characteristic alterations in sleep (Loomis, Harvey, and Hobart, 1937; Blake and Gerard, 1937), so that the depth of sleep can be estimated from minute to minute with considerable accuracy. Figures 2 and 3 show graphically the progress of D.C. potential-differences in 3 experiments and the lack of any relationship to the character of the pattern in the electroencephalogram. In all 3 experiments the subject passed from the waking state (A) to the deepest (E) stage of sleep within a period of an hour and a half or less.

Figure 2 shows the potentials from the entire series of electrodes, referred to the electrode on the left mastoid as reference electrode, determined periodically by the voltmeter in an all-night experiment. The subject was unusually tired. She had had a total of only 22 hours sleep during the 4

previous nights and had driven an automobile 225 miles between noon and 7:30 p.m. on the day of the experiment She reported sleeping soundly all night and was still asleep when the readings were taken at 8:15 a.m. next morning. After breakfast she returned to the experimental room for a second nap. The electrodes remained undisturbed throughout.
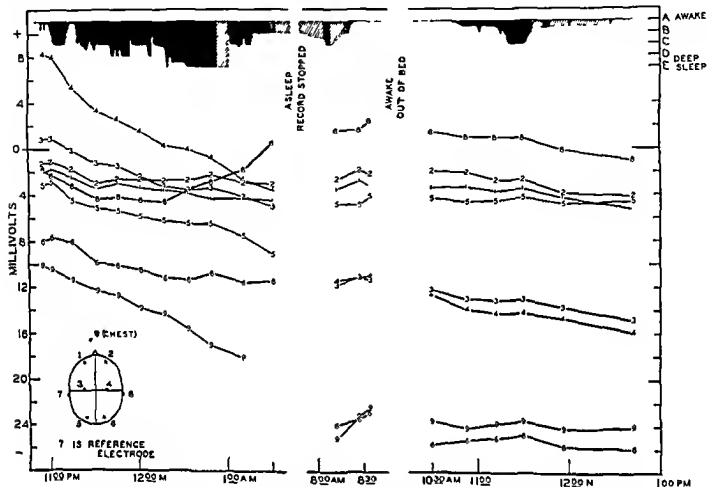

Fig 2 Differences of potential in millivolts measured by balanced D C millivolt-meter between electrodes on head and chest of a 40-year-old woman during the first part of a night's sleep and during a nap the next morning Positions of electrodes are indicated by diagram in lower corner Zn–ZnSO₄-paste electrodes In this and subsequent figures the depth of sleep is evaluated according to the criteria of Loomis, Harvey, and Hobart (1937) from a simultaneous electroencephalogram The record was stopped during the intervals indicated by the gaps and by the shaded areas in the sleep chart

Figure 3A is constructed from the continuous D.C. record of the potential-difference between left occiput and left mastoid taken during the first part of this same experiment. The more rapid initial changes and the steady subsequent drift with minor fluctuations superimposed on it are well illustrated.

Figure 3B is a similar record of the potential-difference between chest and left central area in another subject during the first part of a night's sleep. This subject was also fatigued from driving an automobile continuously for 11 out of the previous 12.5 hours The progressive negative trend of the scalp which occurred in this case did not appear in other experiments.

Figure 3C shows the course of the potential-differences between right

rate of drift which might be maintained steadily for hours (Fig. 2 and 3). Neither the initial shifts nor the more prolonged drifts could be related to the position of the electrode or to any other factor which we have been able to identify.

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Figure 2 shows the potentials from the entire series of electrodes, referred to the electrode on the left mastoid as reference electrode, determined periodically by the voltmeter in an all-night experiment. The subject was unusually tired. She had had a total of only 22 hours sleep during the 4

frontal regions and chest in another subject during an afternoon nap. It illustrates the sudden shift produced by a gross movement and the slow drift back to the original level.

The results of the present investigation fail to disclose any change in D.C. potentials of the head associated with the state of sleep. We plan to explore other parts of the body, especially the palms of the hands and soles of the feet (Forbes and Andrews, 1937), to see whether electrical changes in these other regions can he correlated with states of sleep.

SUMMARY AND CONCLUSIONS

Two methods of measuring D.C. potentials from the human subject are described, one a push-pull vacuum-tube millivoltmeter, the other an adaptation of the usual type of capacity-coupled ink-writing oscillograph. The second method is based upon mechanical interruption of the input potential. The sensitivity of both methods is about 0.05 mV.

Convenient electrodes of the Zn-ZnSO$_4$ and also Cu-CuSO$_4$ types are described. Neither combination is entirely satisfactory, but the errors introduced are small compared with potential-differences arising apparently in the skin.

No correlation could be detected between the stage of sleep and the D.C. potential-differences or changes in D.C. potential observed between chest and head, scalp and mastoid region, frontal and occipital regions, or right and left sides of the head.

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ON THE INFLUENCE OF ANOXIA ON PUPILLARY REFLEXES IN THE RABBIT*

BORIS URY AND ERNST GELLHORN

Departments of Physiology and Psychiatry, College of Medicine, University of Illinois, Chicago

ALTHOUGH EXTENSIVE STUDIES have been published by Gellhorn and collaborators (1934–1937) on the effect of anoxia on cortical phenomena, subcortical reflexes have been but little investigated. In 1935 Gellhorn and Spiesman showed, in experiments on the effect of caloric stimulation of the vestibular apparatus, that under the influence of low oxygen tension nystagmus was decreased. The effects were reversible. It was noted, however, that much lower concentrations of oxygen were needed to bring about these effects than were required for influencing cortical activities. In a recent study Gellhorn and Storm (1938) investigated the influence of anoxia and related conditions on the galvanic nystagmus in the rabbit. They found, in narcotized animals that anoxia predominantly decreases the galvanic response, whereas in the non-narcotized animal an increase of the galvanic response prevails. The results suggest that the effects of oxygen lack on subcortical processes are modified by the cerebral cortex.

It seemed desirable, therefore, to reinvestigate the problem of the effects of anoxia on subcortical structures. In view of the fact that Karplus and Kreidl (1911) assume in the rabbit that pupillary dilatation following pain stimuli is due to an inhibition of the parasympathetic (and not to excitation of the sympathetic), the rabbit was chosen for study. This makes it possible to extend our investigations to other subcortical reflexes and also to include central inhibitory processes in the investigations. Our earlier experiments indicated that relatively small concentrations of CO_2 offset the effects of anoxia (Gellhorn, 1936–37). This was true, not only for cortical but also for subcortical processes (Gellhorn and Storm, 1938). The mechanism involved is largely the synergistic effect exerted by low oxygen and CO_2 tension on the vasomotor apparatus (Gellhorn and Lambert, 1939). Similar experiments on the pupillary reflex dilatation in the rabbit are now reported.

METHOD

Two groups of rabbits were studied: (i) five normal animals; (ii) six animals with unilateral cervical sympathectomy, and one with bilateral sympathectomy. The operation was performed by excision of approximately 1 cm. of the cervical sympathetic in the neck. All animals had a persistent smaller pupil on the operated side under conditions of equal illumination. For the experiments the animals were injected with 0.8 gm. urethane per kg. subcutaneously, and the sciatic nerve was exposed and shielded electrode applied. The preparation was then strapped on to a rabbit board, and the head placed in a modified head holder which permitted the breathing of a gas mixture from a Douglas bag. The

experiment was begun several hours after the injection of the anesthetic and was repeated several times in each preparation

Stimuli were delivered by a Harvard inductorium and one dry cell, the stimulus lasting 3 sec Under these conditions the threshold for the pupillary dilatation lay usually between 11 and 12 cm on the induction coil Occasionally the coil had to be rotated to give a threshold stimulation The eyes were held open by tape, and for 0 5 min preceding the stimulation a strong light was flashed into the eye The diameter of the pupil was measured before and after stimulation by a small microscope fitted with a graduated scale, on which one division represented 0 5 mm in pupillary diameter A pupillary dilatation of 2 divisions was considered threshold, as the stimulus necessary to produce this reaction could be sharply delimited Pupillary reactions below this magnitude gave inconstant values In all experiments the pupillary dilatation was the first sign of the animals' response to the stimulation If stimulation at 9 0 cm gave pupillary dilatation, 8 5 cm gave muscular twitchings, and 8 0 cm vocalization The experiments on the *unilaterally* sympathectomized animals were slightly modified Both eyes were observed simultaneously by two observers and the stimulation time reduced to one second Hereby the determination of the threshold became more precise In order to avoid tilting the induction coil, a resistance was introduced into the stimulating circuit This gave a threshold reaction between 8 and 10 cm. coil distance

RESULTS

The experiments on the influence of anoxia on the pupillary reflex dilatation in normal rabbits are summarized in Table 1. It is seen from this

Table 1 Normal animals

No of expt	Air control threshold	Threshold during O₂-lack	O₂ conc per cent	Air control threshold	Threshold during O₂-lack plus 4 per cent CO_2	Air control threshold
1	8 0 cm *					
2	13 0 cm and 45°	7 0 cm				
3	12 0 cm	12 5 cm	8	8 0 cm	8 0 cm	8 0 cm
4	12 0 cm	10 0 cm	8	13 0 cm and 45°	13 0 cm and 45°	13 0 cm and 45°
5	11 5 cm	10 5 cm	7	12 5 cm	11 5 cm	12 5 cm
6	11 5 cm	10 0 cm	7	12 5 cm	12 5 cm	12 5 cm
7	11 5 cm	12 0 cm	7	11 5 cm	11 5 cm	11 5 cm
8	11 5 cm	9 5 cm	7	13 4 cm and 45°	13 0 cm	13 4 cm
9	10 0 cm	9 5 cm	6 5	11 5 cm	11 0 cm	11 5 cm
		9 0 cm	6 5	11 5 cm	12 0 cm	12 0 cm
			6 5	10 5 cm	10 0 cm	11 0 cm

* Numbers refer to the distance between primary and secondary coil of a Harvard inductorium and indicate the threshold for pupillary reflex dilatation and stimulation of the sciatic

table that in 8 out of 9 experiments the threshold of the reflex pupillary dilatation increases under the influence of oxygen concentrations varying from 6.5 to 8 per cent. On readmission of air approximately the same threshold was observed as prior to the administration of the oxygen-poor gas mixture. The table gives, also, the results of experiments in which the same oxygen concentration was used together with 4–5 per cent CO_2. This experi-

ment was carried out immediately after the first anoxia experiment and was followed by another control in which the pupillary reflex threshold was again determined while the animal inhaled air. Under these conditions (see Table 1)
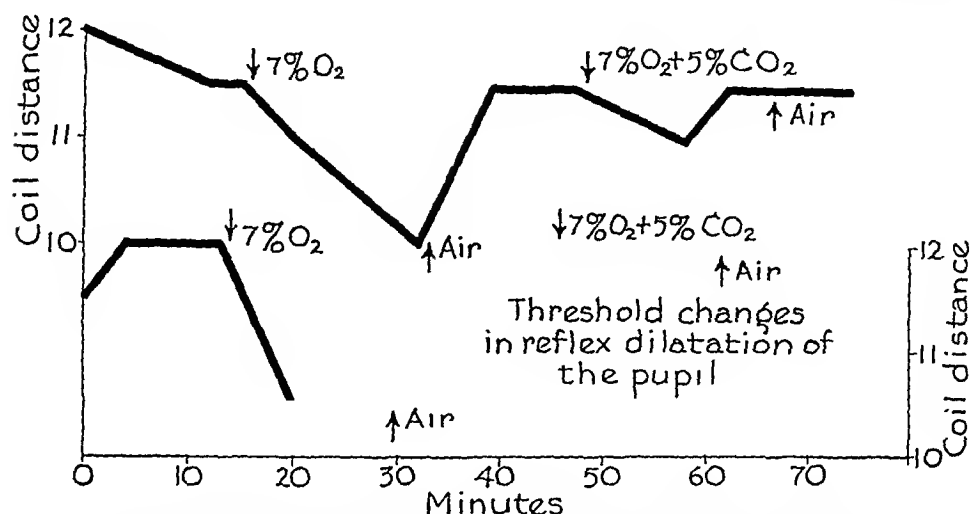

FIG. 1. The effect of 7 per cent O_2 and 7 per cent O_2+5 per cent CO_2 on the threshold of the sciatic nerve for reflex dilatation of the pupil in the rabbit.

the reduced oxygen tension was either without any effect on the reflex threshold, or the reflex threshold was only slightly increased. A graphic record of the 2 types of response is given in Fig. 1. It is evident that CO_2

Table 2. *Unilaterally sympathectomized rabbits.*

No. of expt.	Air control threshold	Threshold during anoxia O_2-lack	O_2 conc per cent	Air control threshold	Threshold during O_2-lack plus 4 per cent CO_2	Air control threshold
1.	8 5 cm.*	6 0 cm.	6 0	7 5 cm.	—	—
2	8.5 cm.	6 0 cm.	6 0	9 5 cm.	—	—
3.	11 0 cm.	10 5 cm.	6 0	11 0 cm.	—	—
4.	9 5 cm.	8 0 cm.	6 0	9 5 cm.	—	—
5	8 5 cm.	6 0 cm.	6 0	8 5 cm.	8 0 cm.	8 0 cm.
6.	8 5 cm.	6 0 cm.	6 0	9 5 cm.	9 5 cm.	9 0 cm.

* All figures refer to the threshold reaction observed on the sympathectomized eye.

either completely or partially offsets the effects of anoxia on the pupillary reflex dilatation.

Table 2 gives the results of experiments on the influence of anoxia on the reflex pupillary dilatation in animals in which the cervical sympathetic had been sectioned on one side. The table shows that the changes in threshold in

the sympathectomized eye under the influence of anoxia are similar to those obtained in the normal eye.

Table 3 gives the detailed record of one experiment in which the pupillary response was noted by two observers simultaneously in the normal and in the sympathectomized eye; there is complete agreement in the reactions. Both the normal and the sympathectomized eye show a gradual increase in threshold with regard to reflex pupillary dilatation during the period of anoxia.

In order to explore the possible role played by the sympathetic system

Table 3 Record of experiment an rabbit with unilateral sympathectomy.

Time	Coil Distance in cm.	Right eye*		Result §	Left Eye	
		D_1†	D_2‡			
10 09	8 0	4	6	+	+	
10 11	9 0	4	4	−	−	Animal
10 13	8 5	4	6	+	+	inhales
10 15	9 0	4	4	−	−	air
10 17	8 5	4	6	+	+	
10.20	8 5	5	5	−	−	
10 22	8 5	5	5	−	−	
10 24	8 0	5	7	+	+	
10 26	8 0	6	7	(+)	(+)	Animal
10 28	8 0	6	6	−	−	inhales
10 30	7 5	6	7	(+)	(+)	6% O_2
10 32	7 5	6	7	(+)	−	
10 39	7 0	5	8	+		
10 41	7 0	5	6	(+)	−	
10.43	6 5	6	8	+	+	
10 46	7 0	7	8	(+)	−	Animal
11:07	8 0	4	8	+	+	inhales
11·09	8 5	5	7	+		air

* Sympathectomized.
† D_1 = diameter before stimulation, 1 unit = 0 5 mm
‡ D_2 = diameter after stimulation
§ + = threshold dilatation = 1 0 mm
(+) = sub-threshold dilatation = 0 5 mm
  − = no dilatation.

the reactions described, 5 experiments were carried out in which first the threshold for the reflex pupillary dilatation was determined, *i.e.*, between 8 and 10 cm. coil distance. Then a drop of 1 per cent homatropin was placed in the normal eye. This abolished the oculomotor tonus and the light reaction in 30 min., while the sympathectomized eye was unaffected. Stimulation of the sciatic nerve for 30 sec. at 0 cm. caused no further dilatation of the atropinized eye, although evoking distinct pain reactions from the animal There was also marked exophthalmos in both eyes. The atropinized pupil could then be further dilated by the instillation of adrenalin and cocaine. Thus no activation of the dilator pupillae was found by impulses passing over the cervical sympathetic in response to peripheral stimulation.

In a last series of experiments the relationship of the reactions to adrenalin secretion was investigated. Two experiments may be cited in which, after the establishment of the reflex threshold, the administration of 6 per cent oxygen, in conjunction with urethane, gave such a deep narcosis that the animal did not give any pain response or reflex pupillary dilatation whatever to sciatic stimulation. Absence of the pain reaction permitted stimulation of the sciatic nerve for 90 sec. with the secondary coil at 0 cm. distance. After that time the sympathectomized eye showed a dilatation of three divisions which took about 2 min. to reach its maximal value and was maintained for 4 min. This reaction differs markedly in latency and speed from the pupillary dilatation in all other experiments in which weak stimuli were used. The slow reaction is obviously due to a humoral excitation of the dilator of the pupil, since the dilatation involving inhibition of the parasympathetic was eliminated by the influence of the narcosis and anoxia.

DISCUSSION

The experiments reported in this paper give conclusive evidence that under the influence of 6–8 per cent oxygen applied for a period of 20 min., the threshold for the pupillary reflex dilatation increases. Furthermore, it was shown that 4–5 per cent CO_2 inhaled simultaneously with a gas with a low oxygen tension offsets to a large extent, or completely, the effects of oxygen lack on the subcortical centers involved. The results are strictly comparable to our observations in humans on the coloric nystagmus and to the experiments on rabbits involving galvanic nystagmus in narcosis. Under the conditions of these experiments in which weak stimuli were given for short periods of time (1–3 sec.) the reaction studied seemed to involve nothing but inhibition of the parasympathetic. This is proved by the fact that the reaction is quantitatively the same in the normal and in the sympathectomized eye. It is furthermore supported by the fact that the stimuli used promptly evoke a dilatation of the sympathectomized eye but fail to do so in the atropinized eye. That the humoral dilatation caused by the liberation of adrenalin plays no part in these experiments is clear not only from the literature, from which it is evident that adrenalin secretion requires stronger stimuli of longer duration (Cannon and Rapport, 1921), but also from our direct observations in which only excessive stimuli applied for more than 1 min. brought about a humoral dilatation of the pupil. The slow onset of the reaction and the slow return of the pupil to its original size distinguishes this reaction fundamentally from those described in this paper.

We therefore conclude that under the conditions of our experiments the reflex dilatation is due solely to an inhibition of the parasympathetic tone of the eye. Summarizing the experiments here described with those reported earlier it may be stated that anoxia diminishes both excitatory and inhibitory processes in the central nervous system.

SUMMARY

Experiments are reported in rabbits on the influence of stimulation of the sciatic with weak faradic currents on the reflex pupillary dilatation. Six to eight per cent oxygen inhaled for a period of 20 min. increases considerably the threshold for the reflex dilatation. The reaction is reversible on administration of air, and is identical in the sympathectomized and normal eye. The simultaneous inhalation of 4 per cent CO_2 prevents the effects of anoxia. An analysis of the conditions of the experiments makes it highly probable that the pupillary reflex studied in this paper involves only the inhibition of the parasympathetic. If this is the case it may be stated that both excitatory and inhibitory processes in the central nervous system are diminished under the influence of anoxia.

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FACTORS CONTROLLING BRAIN POTENTIALS IN THE CAT*

H. H. DUBNER and R. W. GERARD

From the Department of Physiology, University of Chicago

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IT IS NOW ESTABLISHED that central neurones may exhibit spontaneous rhythmic potentials. Even when synaptic transmission is blocked these spontaneous waves continue and may increase in amplitude (Libet and Gerard, 1938). We have studied in the cat some of the physical and chemical factors that influence this "intrinsic" periodicity, as well as the effect on it of external stimulation under varying physico-chemical conditions; certain neural factors affecting the rhythm have also been investigated.

METHOD

The left hemisphere of cats was widely exposed under light nembutal anaesthesia (35 mg. per kg. intraperitoneally), and the Horsley-Clarke instrument attached. An hour later a concentric needle was placed in the desired position along the optic pathways and amplified potentials recorded with a crystograph or cathode ray oscillograph. Retinal stimulation was caused usually by a flash-light directed into the contralateral eye, or sometimes by a single flash of a condenser discharge through a 2.5 V. bulb. A polarizing current (less than 1.0 mA.) was passed through brain tissue between an indifferent silver plate (2 to 3 cm. surface) applied to the surface of the opposite cerebral cortex, and a silver wire (2 to 3 mm. uninsulated tip) placed 3 to 4 mm. lateral to the recording electrode. Solutions injected into the carotid artery or femoral vein were isotonic, neutral, and at body temperature; and those applied to the cortex directly were often buffered with phosphate (this did not alter their action, although it increased control potentials). The various procedures were carried out in irregular order over a period of 10 to 12 hr. Additional anesthetic was sometimes needed towards the end of the experiment. Each agent was tested in not less than 4, usually in 7 to 12 experiments.

RESULTS

Neural factors

Evoked and spontaneous potentials (normal). The responses evoked by directed retinal stimulation (contralateral eye) vary in form and amplitude with the position of the leading-off electrodes, intensity of stimulus, and extent of previous "dark-adaptation." There is also a cyclic fluctuation in threshold, especially after repetitive stimulation (Bishop, 1933). Threshold, latency, and form have been studied by Bartley (1934), Wang (1934), and by Gerard, Marshall, and Saul (1936), and the present description is intended as a norm with which to compare changes. The geniculate "on" response consists of an initial negative potential (50 to 90 μV.) followed by a prolonged positive wave (Fig. 1B, 2 and 3A). Superimposed upon the latter are often additional positive waves which increase with heightened general excitation (Fig. 4A). The "off" is similar in form and direction, but 20 to 50 μV. less in amplitude. Repeated illuminations evoke in the geniculate, as

in the radiations and cortex (Bartley, 1936), responses which show a semi-rhythmic fluctuation in amplitude and form. "Off" potentials tend to increase on repetition, and the "on" spike may give way to several positive waves. When spontaneous potentials are marked, the responses to light are not discernible, although 1 sec. later they may be strong (Fig. 1Ac).

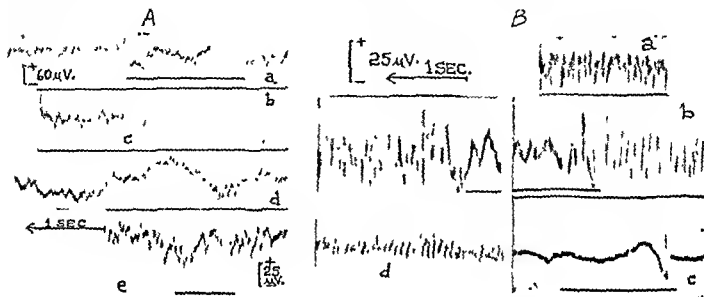Throughout the optic pathways a regular rhythm of from 2 to 4 per sec. is present, which usually becomes asynchronous during retinal stimula-

FIG. 1A. Spontaneous and evoked potentials from the lateral geniculate body. After two hours in dark: (a) Response to illumination (black line). Note the initial increase and later suppression of the rapid waves present before illumination; (b) 5 sec. later, note the rhythm at 4.2 a sec.; (c) 10 sec. after (b), rhythm now 2.7 a sec.; (d) moderate diffuse constant illumination, note alternate periods with and without rapid waves; (e) electrode 3 mm. higher, usual "on" and "off" optic response.

FIG. 1B. Lateral geniculate potentials following brain section: (a) Following ipsilateral decortication; (b) 5 min. after subsequent midbrain transection, note greatly increased fast and slow waves, much reduced during illumination; (c) 4 min. after additional nembutal, optic stimulus; (d) 1 hr. after (c).

tion (Gerard, Marshall and Saul, 1936). This rhythm is most prominent with optimum afferent excitation, and was best studied by continuous observations for 6 to 10 hr. at a given locus. After 1 to 2 hr. of absolute darkness, the 2 to 4 per sec. rhythm was often replaced by continuous, irregular, fairly rapid waves. A directed light then gave "on" and "off" responses and inhibited the high frequency, but it did not initiate a rhythm. In diffuse light of moderate intensity (ceiling light on), however, the rhythm reappeared in 4 to 6 sec. This rhythm could be abolished by retinal stimulation with focussed light. The slow rhythm has superimposed on it waves at 8, 20 to 25, and 60 to 80 per sec., all of which are inhibited during light. The 8 and 20 per sec. waves are present only in the lateral geniculate and radiations, and are normally small, 15 and 5 μV. respectively. They are considerably augmented by increased blood potassium, by strychnine and polarization, and during the after-discharge from retinal stimulation. The 60 per sec. waves of 5 to 10 μV. are most prominent in the large ventral cells of the lateral geniculate and fade off in the optic tract and radiations. They persist

after ipsilateral decortication and are markedly augmented following mid-brain transection (Fig. 1B), though still eliminated by light directed especially into the contralateral eye. Only under optimal conditions are the slow and the high frequency rhythms distinct in the distal radiations and cortex.

Following a retinal stimulus and the "off" response, the after-discharge modifies for some time the continuous activity. This effect varies with the previous degree of dark adaptation and the number of preceding flashes of light. After a single flash the 3 and 60 per sec. geniculate rhythms return within 5 sec., but following a second flash a high frequency after-discharge develops which lasts 15 to 20 sec. When the slow rhythm returns, it may be half again as fast as normal, *e.g.*, 4.2 per sec. 5 sec. after "off," and 2.7 after another 10 sec. Repeated stimuli (under light anesthesia) induce a regular alternation, at about 1 sec. intervals, with the 60 per sec. rhythm now absent, now present, on the continuing slow waves. This alternation lasts longer after many flashes than after few, but it finally returns to the initial resting state with both rhythms present. Under deeper narcosis, a similar alternation may appear during continuous diffuse illumination of moderate intensity (Fig. 1A a to d), to be inhibited by light directed into the eyes. In the optic tracts, in contrast to the geniculate, the slow rhythm returns almost immediately even after repeated light flashes.

Potentials following section of the brain. Bishop (1933, 1936) attributed the slow rhythm of the optic pathways to reverberating thalamo-cortico-thalamic circuits (Lorente de Nó, 1934, 1935). Interruption of these pathways by uni- or by bilateral removal of the parietal and occipital cortex does not abolish the geniculate rhythm in the cat (Fig. 1Ba). Complete mid-brain transection (Fig. 1Bb), caudal to the colliculi, regularizes the slow rhythm and doubles its amplitude, to 40 μV., and strikingly augments the the 25 per sec. waves, to 25 μV. These modified rhythms persist for hours; are completely inhibited by light directed into the eyes, and return following it; and they are depressed to less than one-fourth their amplitude within 1 min. of the injection of additional nembutal (15 mg. per kg.), gradually to regain full size as the anesthetic wears off.

Physical factors

Brain polarization. A feeble constant current passed through the brain and concentrated toward a "different" needle electrode, placed just lateral to the pick-up electrode, enormously increases the amplitude of potentials in the optic pathways, especially the lateral geniculate, usually with no change in rate (Fig. 2). The direction of the polarizing current is immaterial, although perhaps the different electrode as anode favored the appearance of waves of high frequency. The augmentation may persist for 10 min. following a 30 sec. polarization. Besides the great increase in the slow geniculate rhythm, the "on" and "off" responses to light are also intensified and sometimes are followed by high frequency discharges. The possibility that these changes are an artefact due to the polarizing current is excluded by

the following observations: the induced increase in the rhythm depends on the brain structure and is very marked only in the geniculate, the effects long outlast the polarization, and at a given locus polarization may slightly or greatly increase the rhythm, depending on the state of the animal and other physiological variables.
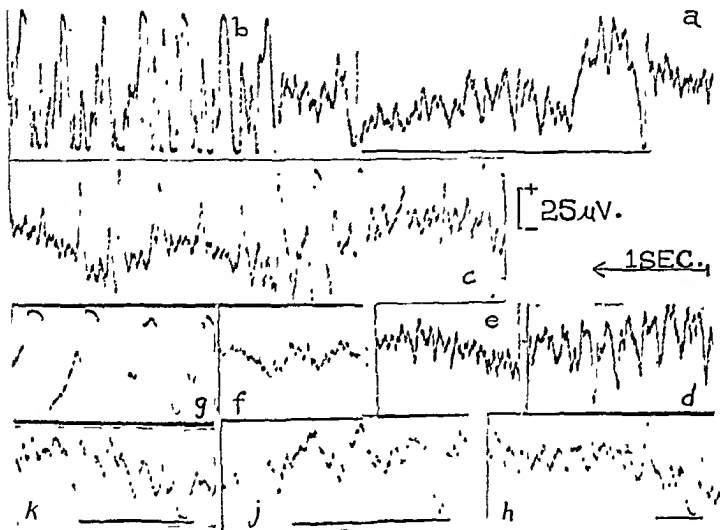

FIG. 2. Potentials modified by brain polarization. Lateral geniculate: (a) Control with light response; (b) during polarization, amplifier overloaded; (c) 10 sec. after polarization; (d) and (e) 2.5 and 13 min. after polarization, respectively. Another cat: (f) control; (g) during polarization. Electrode to optic radiations; (h) control and optic response; (j) during polarization, note rapid waves at "off"; and (k) 10 sec. after polarization.

Chemical factors

Potassium ion. Intravascular injection of isotonic KCl, sufficient to increase the blood potassium from 20 to 50 per cent above normal, induced psychomotor excitation with: gasping respiration, increased deep reflexes, occasional bilateral running movements, urination, loud vocalization, and purposeful scratching gestures. Within 2 min., the slow geniculate rhythm and, especially, the superposed 8 per sec. waves were augmented. When these had again diminished, a retinal stimulus still increased them; 5 min. after injection a continued retinal stimulus gave a somewhat increased "on" response followed by regular 45 per sec. potentials which waxed and waned at 2 per sec. (the frequency of the usual slow rhythm; Fig. 3A). Potentials

of the optic cortex were not clearly affected. Within 10 min. the animal returned to surgical narcosis and the thalamic potentials resumed their pre-injection character. These time relations parallel those of rise in blood po-
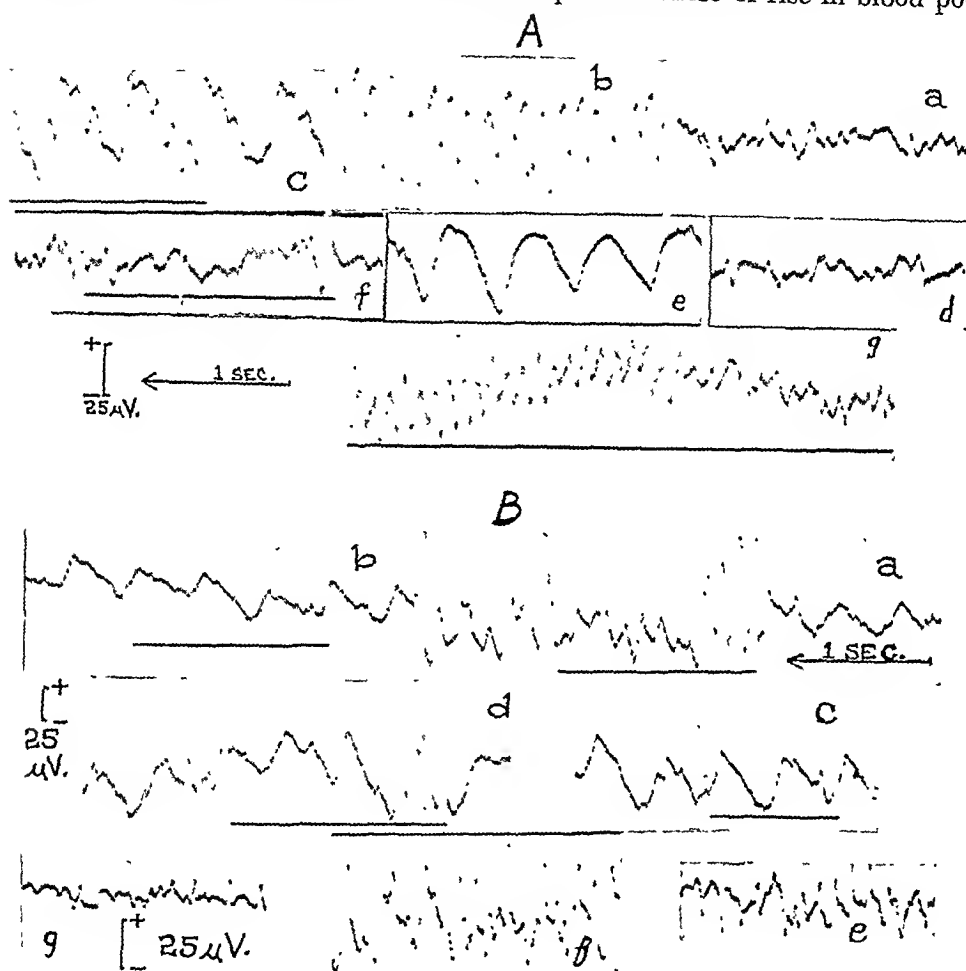

FIG. 3A. Lateral geniculate potentials as modified by ions and strychnine on intra-vascular injection. (a) Ringer's solution injected as control; (b) 2 min. after K; (c) 30 sec. after (b); (d) 30 min. later; (e) 2 min. after Ca; (f) 15 min. after (e); (g) 4 min. after strychnine.

FIG. 3B. Striate cortex potentials (surface record) as modified by ions locally: (a) Normal rhythm and optic response; (b) 10 min. after K; (c) 10 min. after (b) and washing with Ringer's solution, note limited recovery; (d) 1 min. after (c) and after washing with Ca. Another animal: (e) 10 min. after K; (f) 15 min. after (e) and after washing with Ringer's solution; (g) 2 min. after Ca.

tassium following intravenous potassium administration (Houssay and Marenzi, 1937). The largest single intravenous dose of KCl tolerated was 15 mg. per kg. in cats; Houssay and Marenzi found 20 mg. per kg. in dogs. Death was apparently due to cardiac failure.

Local application of excess K ion to the striate cortex reversibly depresses and may completely abolish spontaneous and evoked potentials. (With sufficiently weak potassium solutions, the expected increase of potentials is obtained.) Ringer's solution with tripled K ion cuts the amplitude to half in 2 min. with spontaneous recovery in 7 to 10 min. Isotonic KCl completely abolishes the normal "on" and "off" potentials (initially 100 μV.) by 2 to 10 min. after application (Fig. 3Ba and b). There is little return in another 10 min. even after washing with warm Ringer's solution (c); but application of isotonic $CaCl_2$ restores normal responses within a minute (d). The spontaneous rhythms are similarly suppressed by potassium excess and restored by calcium. Potassium may also reverse the polarity of the waves (Fig. 3Be).

Calcium ion. Intravascular injection of isotonic $CaCl_2$, sufficient to increase the blood concentration 50 to 200 per cent, depresses the animal, if under light anesthesia. Within 2 min. the fast irregular geniculate potentials disappear, the 2 per sec. rhythm is regularized and enhanced, and the amplitude and duration of the after-discharge to light is decreased (Fig. 3Ad and e). The increased and stabilized slow rhythm now persists during optic stimulation, which normally disrupts it. Intravascular Ca ion excess was found to be less toxic than K ion excess, and calcium action could be tested within 20 min. of a preceding potassium injection.

Local application to the optic cortex of Ringer's solution with three times the normal calcium does not alter potentials, but isotonic $CaCl_2$ depresses evoked and spontaneous ones during 10 min. (Fig. 3Bf and g). The calcium depression is antagonized by potassium or by citrate, which promptly restores normal responses. Calcium ion deficiency, produced by intravascular injection of 2.0 cc. of 3.0 per cent Na citrate in Ringer's solution, acts upon lateral geniculate potentials much like potassium excess.

Hydrogen ion. Intravascular injection during 30 sec., of 1.0 cc. per kg. of 0.01 N HCl in Ringer's solution increases respiratory depth and evokes general restlessness and movement. It also disrupts the slow lateral geniculate rhythm, decreases the amplitude of spontaneous and evoked potentials, and prolongs the after-discharge. The rhythm does not reappear for 20 to 30 min. Local application of approximately 0.001 N HCl in Ringer's solution (unbuffered) evokes, within 2 min., wave trains at 7 to 12 per sec. and 40 to 60 μV. These reappear during retinal stimulation after the initial effect has worn off.

An *increase* of pH is more toxic than a decrease, but less prolonged in action. Rapid injection of 2 cc. of 0.01 N acid or 1.5 cc. of 0.001 N alkali per kg. is usually fatal. (Barbitalized dogs can tolerate as much as 3 cc. of N HCl injected slowly during 3 min., Harkins and Hastings, 1931; the maximal pH fall, to 6.92, occurs in 6 min.) Smaller amounts of NaOH depress respiration and general motor activity, immediately disrupt the slow geniculate rhythm, and markedly diminish the spontaneous and evoked poten-

tials. Normal potentials return in 6 to 8 min. At these pH extremes, acid and alkali injections do not consistently counteract one another.

Lactate ion. Less than 2 min. after the intravascular injection of 2.0 cc. of 4 per cent Na lactate, the slow geniculate rhythm is less regular and smaller and is largely replaced by rapid, large, irregular potentials. These are especially prominent in the after-discharge following optic stimulation. The modified potentials persist for 1 to 2 hr.

Strychnine. Within 1 min. after the intravascular injection of 2.0 cc. of 1.0 per cent strychnine solution, the slow geniculate rhythm is augmented; often, when no obvious rhythm is present in a dark-adapted cat, strychnine initiates conspicuous rhythmic potentials. During optic stimulation the augmented slow rhythm is replaced by waves at 13 per sec. and 15 μV., to reappear almost immediately after the "off" (Fig. 3Af and g). When some minutes have elapsed following the strychnine injection, the rapid rhythm appears during illumination and continues as an after-discharge. The high frequency potentials of potassium excess, in contrast, appear primarily during light and less frequently in the after-discharge.

Blood sugar. Nembutal anesthesia causes no change in mean blood sugar level (Hrubetz and Blackberg, 1938). Insulin, in a dose of 10 to 15 units per kg. subcutaneously, does not lead to gross convulsions, though geniculate potential changes are definite within an hour. (Compare the time course of blood sugar changes; Zucker and Berg, 1937. Smaller insulin injections do not affect potentials.) The background potentials become augmented and the normal diphasic "on" and "off" responses to light are replaced by 3 to 5 large oscillations, mainly positive, followed by a prolonged high-frequency after-discharge (Fig. 4A). The picture is similar to that induced by potassium excess. Similar quantities of insulin, given intravenously, lead to repetitive optic responses, but they markedly decrease the spontaneous potentials (Fig. 4B). In both cases, the altered responses to light remain constant with repeated stimuli. The normal picture is restored within 5 min. by intravascular glucose (2 cc. of a 40 per cent solution). Hyperglycaemia leads after some 10 min. to an increase in rate and amplitude of the slow rhythm (Fig. 4Ag and h).

Hypoxia. Bilateral occlusion, by traction, of the carotid arteries reversibly abolishes the spontaneous and evoked potentials in the lateral geniculate body. Electrical activity disappears usually in about 1 min., and returns in 1 to 3 min. after release. Optic responses are lost at the same time as the spontaneous ones. In 3 animals with transected brain stem (and loss of vertebral blood) the average times of loss and return of potential were 50 sec. and 80 sec., as compared with 240 and 400 sec. in 3 intact animals. Before anoxic failure and early during subsequent recovery, potentials are increased, with a large high-frequency component.

DISCUSSION

The unevoked potential rhythms of central neurones are controlled by their own states of metabolism, membrane charge, etc., which in turn are
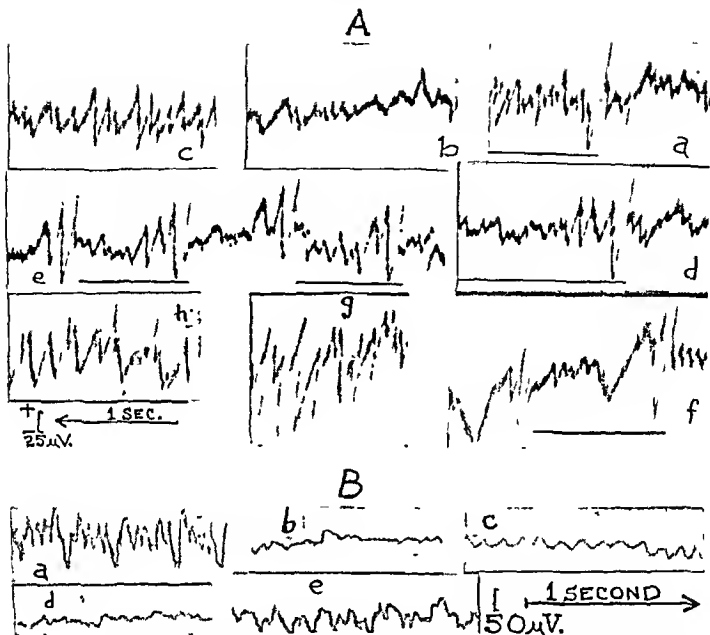
FIG. 4A. Lateral geniculate potentials as altered by change in blood sugar: (a) Normal "on"; (b) 2 sec. and (c) 10 sec. after "off"; (d) 20 min. after insulin (10 units subcut.) single "on" and (e) repeated optic stimuli, note multiple waves; (f) 6 min. after (e) and intravenous glucose, "on" and "off"; (g) 2 and (h) 20 sec. after (f).

FIG. 4B. Like Figure 4A, undulator ink records obtained at Ohio State University. (a) Before insulin; (b) 70 min. after intravenous insulin, dark, (c) during light, (d) dark, 2 min. later; (e) 8 min. later, following intravenous glucose.

modified by influent nerve inpulses and the condition of the surrounding medium. As recorded, these potentials involve the further factors of the number, kind, and distribution of active cells or cell groups and the degree of unison of their separate beats. Interpretation of such records at present remains rather circumstantial for the mammalian brain. Cells of the lateral geniculate nucleus are roughly stratified with the largest ones located most ventrally. Of the four more or less distinct rhythms, the faster are more prominent in the ventral portion. Perhaps the larger cells normally manifest a faster rhythm, but at all needle positions there is considerable play of potential rate from time to time under seemingly fixed conditions.

The slow 3 per sec. rhythm, seen especially in the geniculate, might arise in the eye and be normally imposed on other portions of the optic system.

It is seen in the primary tract and, after abolition by light, reappears in it earlier than in the geniculate. On the other hand, the geniculate rhythm persists for some hours after severing the optic nerves, so that the thalamic cells can maintain the beat alone. It similarly survives, is even enhanced by, the removal of the optic cortex, so that any thalamo-cortical cycle which may exist is not essential. The same is true for connections with the mesencephalon, section of them favors the diencephalic rhythm (see also Bremer, 1935), and even full synaptic blockade with nicotine leaves the geniculate active for a time (Libet and Gerard, 1938). These neurones, then, do not depend on immediate nerve impulses from eye, cortex, or brain stem to maintain their beat.

Over longer periods this may not be true; also, at any given moment, the local behavior is modifiable by incoming impulses. The absence of the rhythm after prolonged dark and its return in moderate diffuse light can be interpreted in terms of a state of cell excitation which slowly falls below threshold for the rhythm when no afferent impulses are being received. Any enhancement of the general level of cell excitation (whatever the metabolic, membrane, or other mechanism for controlling this level may be) favors the potential waves; and they are increased during diffuse and after strong optic stimulation and by potassium and strychnine.

The disruption of the rhythm during strong light has been generally interpreted as a desynchronization of the many cells, rather than as a decrease in the beat of each. The effect of polarization is presumably just the reverse of this. A rhythm, initially feeble and more or less obscured by the partial asynchrony of cells, is greatly increased and regularized (but not altered in form or rate), as an imposed potential sweeps the cells into unison. The change is independent of the direction of the imposed current, which in any event flows across irregularly oriented cell processes, and outlasts the current for many minutes. The current may, of course, alter the beat of individual cells as well as their phase relations. On this there is no evidence except that a cell stimulation would probably increase rate, which occurred in only one case.

The influence of potassium increase and calcium increase or decrease (citrate), on optic potentials is in accord with their action on neurone metabolism and activity (see Gerard, 1938). Moderate increase of potassium enhances irritability and excitation, and it augments potentials, especially the faster rhythms. Greater concentrations of potassium, applied locally to the cortex, depress irritability and potentials. Calcium decrease acts similarly, excess oppositely, and increase of both potassium and calcium antagonizes the action of excess of either alone. After-discharge following optic stimulation is prolonged by potassium, shortened by calcium. (Cf. motor discharge after cerebellar stimulation; Gerard and Magoun, 1937.) The dominant 3 per sec. wave of the geniculate is not abolished by calcium injections but, due to suppression of faster waves, remains more clear than normal. It may be actually strengthened, or at least cell synchrony made more positive, by increased calcium since light no longer disrupts it. Possibly

cells, which at "normal" excitation levels produce the faster rhythms, fall into the slower tempo when somewhat depressed by calcium.

The marked increase and decrease of pH studied here both disrupt the slow rhythm. Acidity increases the 8 per sec. waves and prolongs after-discharge, alkalinity depresses all waves, including evoked potentials and their after-discharge. Over a finer range, cortical potentials are increased by an alkaline shift in the cortex and diminished by an acid one (Dusser de Barenne, McCulloch, and Nims, 1937), in parallel to changes in irritability. The action of strychnine (by injection), in initiating or increasing a regular slow rhythm and in making more rapid and intense the after-discharge following physiological stimulation, requires no comment. Strychnine spikes were not seen in any of these experiments as on local application. (See Jasper, 1937; Dusser de Barenne and McCulloch, 1938.)

Of especial interest are the effects of hypoglycaemia (insulin) and of glucose injection. Others have reported, for the cortex, mainly of human subjects, a slowing of the normal rhythms under insulin action (Hoagland, Rubin and Cameron, 1937; Lennox, Gibbs, and Gibbs, 1938). The "delta index," of slow wave components, inversely follows blood sugar concentration, sometimes quite accurately. Such slowing has been interpreted as evidence of a decreased metabolism of the active neurones. On the other hand, hypoglycaemia acts much like hypoxia, which induces increased neural sensitivity and discharge as a transient phase between the normal and depressed states. (Gerard, 1938.) The present findings for the geniculate indicate such an excitation. Under insulin action (and promptly abolished by glucose) the spontaneous rhythm is enhanced, the "on" and "off" light responses are larger than and show complex potential swings not present in the normal ones, and the after-discharge is more prolonged and of a higher frequency. Hypoglycaemia, in fact, affects geniculate potentials much as does increased blood potassium—even to a reversal of the excitant action when concentration changes are excessive. Insulin causes a decrease in blood potassium (Keyes, 1938) while anoxia causes an increase (Mullin, Dennis and Calvin, 1938). In the latter case the potassium content of the brain is, if anything, increased (Gerard and Tupikova, unpublished), the potassium entering into circulation from other sources. Under insulin, also, it is possible that brain potassium is increased, some entering from the blood. This has yet to be determined. By whatever means low blood or brain carbohydrate produces its action, it does seem that it is able to increase brain activity as well as to depress it.

SUMMARY

The normal spontaneous rhythms of the cat's geniculate body, especially the dominant one at 3 per sec., are independent of impulses reaching these neurones from optic nerves, cortex or brain stem. The background level of excitation, determined largely by optic impulses, however, strongly influences their character. The slow rhythm fades out over hours in the dark and is reinitiated after brief illumination.

The enhanced spontaneous and evoked optic potentials induced by potassium, citrate, acid, strychnine, insulin and polarizing currents, and the diminished potentials resulting from calcium, alkali and glucose are described and interpreted.

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CONTROL OF THE POTENTIAL RHYTHM OF THE ISOLATED FROG BRAIN*

B LIBET AND R W GERARD

From the Department of Physiology, University of Chicago

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INTRODUCTION

THE PRESENCE of "spontaneous" rhythmic electric potentials in groups of nerve cells has been observed by most students of electrophysiology. These potentials exist in the absence of deliberately evoked nervous impulses (Berger, 1929; Bartley and Bishop, 1933; Gerard, Marshall and Saul, 1936; and others) and they persist, even for hours (Gerard and Young, 1937), in isolated groups of nerve cells (Adrian, 1931; Adrian and Buytendijk, 1931) despite the blocking of synaptic conduction by nicotine (Libet and Gerard, 1938). Such facts give impressive evidence that the rhythms are truly spontaneous, in the sense that no nerve impulses are immediately necessary to their evocation.

Several laboratories have investigated the physico-chemical factors influencing these rhythms in man and laboratory mammals (Dubner and Gerard, 1936 and 1939; Hoagland, 1936; Hoagland et al., 1937; Lennox et al., 1938; Jasper, 1936; Davis, 1936; see also Prosser, 1933, on the crayfish). The present experiments, directed to the same end, have been carried out under the much simpler conditions offered by the isolated frog brain. Information has also accumulated on the influence of incoming nerve impulses on cell potentials and concerning the mechanisms of synchronization of cell groups.

METHODS

Preparation The brains of grass frogs (R pipiens and R foxinus) and bullfrogs (R catesbiana) were used Results were similar for all, freshly captured animals yielding the best preparations Potentials from the brains of bullfrogs average perhaps twice the amplitude of those of the smaller frogs, although some of the latter have shown the greatest activity, and bullfrogs were used for experiments on the olfactory nerves because of their larger structures The brain can be exposed and removed either from the separated head (decapitation without anesthesia) or from the intact etherized animal Decapitation is accomplished by a single snip of a pair of scissors at the angles of the mouth, with the top blade as far caudalward as possible The dorsal skin is reflected and, with the eye and muscles, trimmed off on one side The skull is rapidly twisted outward with sturdy forceps from the open rear end forward, the brain gently lifted to one side, and the cranial nerves, including the olfactory, cut through The freed brain is lifted with a small spatula or forceps and placed in Ringer's solution in a small glass container The dura mater often slides off as the brain is lifted with forceps, if not it is carefully cut away Pressure or tension, especially on the olfactory bulbs, must be carefully avoided The original decapitation must be by shearing rather than crushing and the olfactory nerves severed (with fine scissors slipped forward under the brain) before lifting the brain After some practice, this whole procedure requires less than 2 min and the isolated preparation manifests potential rhythms almost without exception This procedure is not applicable to the bullfrog, with tougher bony plates, and the brain is exposed in the usual manner in the etherized animal It is then covered with cotton soaked in Ringer's solution and the frog allowed to

blow off the ether for 1.5 to 2 hr.; after which time the dura is reflected, the brain sectioned in front of the optic lobes, the olfactory, optic and oculomotor nerves cut, and the fore-piece of brain removed to the container. Grass frog brains give like results when removed by decapitation or after etherization. Occasionally, recordings were made from the exposed intact brain *in situ* with the animal immobilized.

Electrodes. Ag–AgCl-wick electrodes (Adrian, 1936) were used. Silver tubes (0.3 mm. internal diameter, 15 mm. long), attached to solder wires for separate adjustment of each electrode, were held in a clamp with universal adjustment. A Ringer-soaked cotton thread, 0.2 mm. in diameter and projecting 1 to 2 mm. from the tube, served as wick. With this in place, a current of 1.0 mA. for 2 hr. deposited AgCl between tube and thread giving an electrode of some 10,000 Ω. resistance. During 3 or 4 weeks, although stored in Ringer's solution, resistance rises to 30,000 Ω, when the electrode is discarded. One wick was regularly placed on an olfactory bulb, the other 10 mm. lateral in Ringer's solution (or on bone). During the recording, the solution was sucked away so that only a film connected brain and indifferent electrode. It was found possible repeatedly to reset electrodes and to replace and remove solution with no significant change in the amplitude of recorded potentials; so that physical short-circuiting was constant and physiological injury absent. The

Table 1.

mM/liter	Frog plasma (Fenn)	Ringer used
K	2.5	1.9
Na	103.8	111.0
Mg	3.0	—
Ca	2.0	1.1
Cl	74.3	114.0
HCO₃	25.4	—
Lactate	3.3	—
Phosphate	3.1	—
	215	228

electrode was lowered just until the fluid on the brain and wick coalesced, for pressure soon disrupted the slow rhythm. Grounding the indifferent lead had no effect. Fine platinum electrodes (0.3 mm.) were sometimes used, with one or both placed on or in the olfactory bulb. These yielded larger potentials but less regular and less constant ones, due to pressure injury, and were employed only for special purposes.

Recording. The push-pull, resistance-capacity coupled amplifiers, cathode ray oscillograph and crystograph were used according to standard practice in this laboratory. The usual controls of the electrical system, e.g., with electrodes on dead brain, established the validity of observations on the living tissue.

Solutions. The ionic composition of Ringer's solution (Starling, 1936) differed somewhat from that of frog plasma (Fenn, 1936; Table 1). Since addition of carbonate or phosphate in the amounts present in plasma did not affect results, their absence is immaterial. Phosphate buffer (pH 7.4, 1/75 or 1/150 M) was also without influence and, therefore, usually omitted when not required. Magnesium, in the concentration normally present, does affect potentials (see below), and its absence from the Ringer's solution may have modified the findings; the higher ratio of K to Ca + Mg in our solution than in plasma perhaps contributing to the final disruption of the rhythm; but the saline solution as used would not have masked the action of the added ions since their effects increased with the concentration and were also reversible. Substances to be tested were added to the Ringer's solution without equivalent removal of ions; the slight increase in osmotic pressure so produced being well below that required to alter potentials. For pH values between 6.0 and 7.8, phosphate buffer was used; below 6.0, acetate; and above 7.8, borate (with NaCl replacing KCl in the buffer). The final pH was checked to 0.02 units with glass or hydrogen electrodes. No calcium precipitated, even after long standing of the buffers.

The solution surrounding the brain was changed by a standard procedure, to favor constant diffusion and other conditions of action of the substance tested. The electrodes

were raised, the solution sucked out, the brain and electrodes washed twice by gentle flooding with new solution and then left covered by it for 3 min.; finally the fluid was sucked out and the electrodes lowered into place. Replacing old Ringer's solution with fresh, to control the procedure, caused no change in potentials.

Temperature. For most experiments, room temperature, 22°C., was sufficiently constant. When studying temperature itself, the brain container was submerged almost to the rim in water in a Dewar flask and the brain nearly covered with saline at the same temperature. One junction of a thermocouple, lagged thermally with paraffin, hung into the water, the other was inserted into a cerebral hemisphere posterior to the bulbs. The water temperature was directly measured to 0.1°C. and did not change this much during a single run. Any difference between the temperature of the brain and the water was measured via the thermocouple to 0.05°C., a single determination requiring less than 10 sec. The actual temperature of the cerebral hemisphere was thus accurately followed as the system was changed from one temperature to another. Since electrical records were obtained from the olfactory bulb, with a wick electrode conducting heat, rather than from the hemisphere, where temperature was measured with 38-gauge wire conductor, other controls (dead brain, electrode and thermocouple together on bulb, etc.) were made to test the temperature comparability. No difference was seen in the rate of attaining temperature equilibrium by the two brain regions. The maximal temperature error possible in these measurements, 0.1°C., would not alter the estimated Q_{10} by 1 per cent.

Stimulation. Olfactory nerves were sometimes stimulated by induction shocks via platinum electrodes fixed in the container. The primary was fed waves at 40 to 60 per sec. from a thyratron. Stimulus artifact could not be eliminated but recording was started in less than 1 sec. after the end of tetanization.

RESULTS

Experiments on over 200 brains were directed to the study of such basic influences as temperature, salt and hydrogen ions, etc., on potentials. Such information is valuable in itself, and is also necessary to the examination of the action of more specific substances to be reported later.

Spontaneous rhythm

In vivo. The exposed uninjured olfactory bulbs of a frog, after recovery from anesthesia, exhibit a basic rhythm which is ordinarily constant in frequency and amplitude in any individual to within 5 per cent, irregularity being partly due to variation in superposed higher frequency waves. The fundamental frequency averages 6.0 per sec., extremes 4.0 and 8.5, with an average amplitude of 30 μV., extremes 10 and 40. The whole surface of the bulb is electrically active. Exploration with a fine electrode (*in situ* and after isolation) shows the greatest and most regular potentials on the free lateral aspect and on the dorso-medial surface; the dorsal aspect between these is less active and the ventral still less. Electrodes on both bulbs record the same potentials as an active electrode on one and an indifferent electrode elsewhere, showing lack of electrical synchrony of the two sides. The faster feebler rhythms encountered in other parts of the brain (Gerard and Young, 1937) have not been further examined.

In vitro. Immediately after isolation of the brain, the olfactory bulb rhythm is much stronger, average 100 μV., and more regular in height and wave form. Its average frequency is not altered but the moment-to-moment variability is decreased, from 5 per cent to 2 (Fig. 1A a and b). This powerful beat continues in the absence of measurable impulses from the olfactory

nerve or the remaining brain; for the former shows no action potentials and the electrical record from the hemisphere is flat in 15 to 20 min. after isolation, that from the optic lobe in 30 min. The bulb itself may continue to
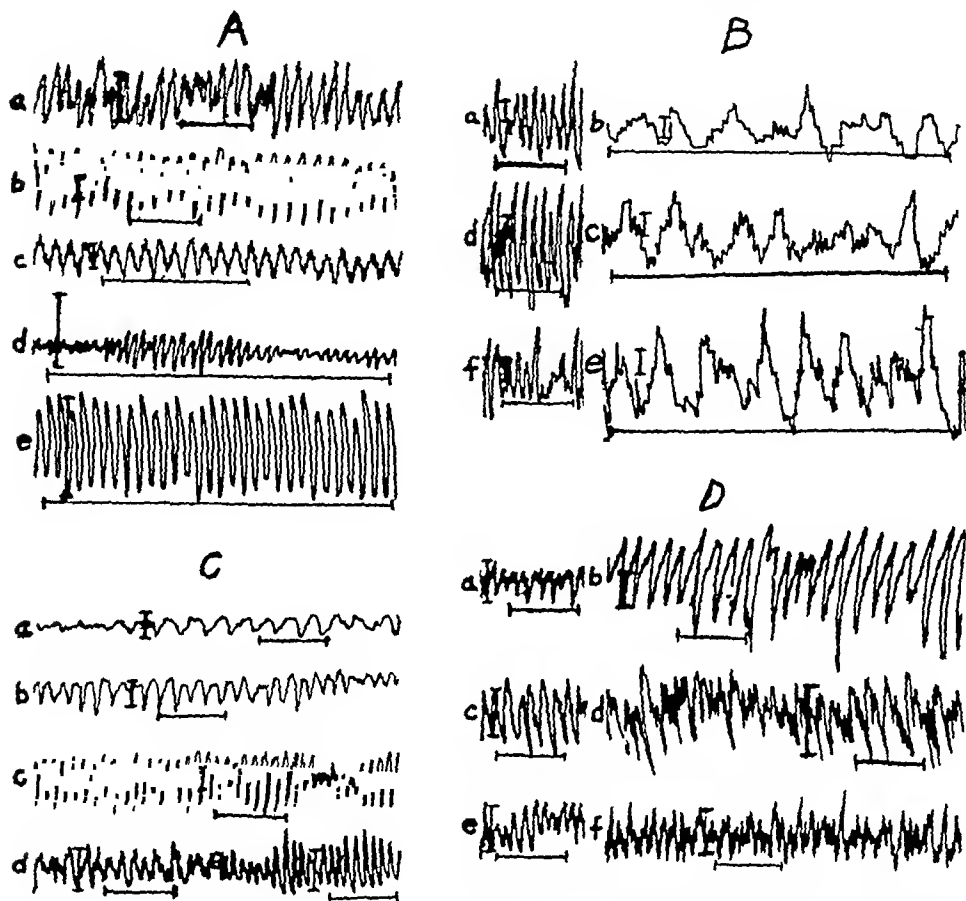

FIG. 1. A. a. Olfactory bulb *in situ.* b. Same bulb 1 min. after isolation of brain. c. Two bulbs separated from each other but attached to hemisphere. d. Single separated bulb. e. Single bulb fragment, about 0.1 mg.

B. a and b. Normal. c. 3 sec. after a 12-sec. stimulation of the olfactory nerves. d, e, f, 4.5, 6.5 and 13 min. after stimulation.

C. Bulb at: a. 15°C.; b. 22°C.; and c. 33°C. Another at: d. 22°C.; e. 28°C.

D. a, c, e, normal controls, each a separate brain; b, d, f. after 5 min. in test solutions. b. Ringer's plus glucose to double osmotic pressure. d. Ringer's plus acetate buffer, pH 5.5. f. Ringer's plus borate buffer, pH 9.0. Electrical records in all figures are from the olfactory bulb of an isolated brain except as indicated. The horizontal straight line represents one second, read left to right, the vertical one, 30 µV, in all cases.

give potentials for 3 hr. or more, even with no special handling. Often after 10 to 15 min. the steady rhythm begins to wax and wane, and after 30 to 60 min. individual waves become somewhat irregular. Irregularity slowly increases and one or more faster rhythms (10 to 45 per sec.) come to supplant

the usual one. Amplitude falls with increased frequency and decreased regularity until the waves finally fade into the baseline. If frog serum is used in place of Ringer's solution, the 6 per sec. rhythm is retained longer (60-90 min.), irregularity is not so marked, and activity is not finally lost until some 4 hr. after isolation.

Further separation of the bulbs, by cutting away the hemispheres with a razor, results in inactivity, due to pressure injury by the blade. Such pressure was avoided by separating the brain tissue with two pairs of finely ground forceps, the tissue not immediately about the instrument thus being spared mechanical insult. In similar fashion, the two bulbs were later separated from each other, and even a single bulb picked to pieces. Each successive step led, in the majority of cases, to a discontinuous increase in frequency. Thus, for example, removing the hemispheres raised the frequency from 6 to 8 per sec.; separating the two bulbs raised it to 10 per sec.; isolating a bulb completely, to 30 to 40 per sec.; and, finally, isolated bulb fragments of about 0.1 mg. gave a regular rhythm, e.g., at 30 per sec. and of 15 μV. intensity (Fig. 1A c, d, e). There was usually a corresponding loss in amplitude, but occasionally an increase occurred in this as well as in the frequency. In two instances, bulbs which had ceased activity regained it after separation from the hemispheres; and, in another case, activity was enhanced at once on separation, and continued to increase further during several minutes.

Effects of stimulation

The return or increase of olfactory bulb potentials produced by isolation procedures must be due to removal of inhibitory control or to direct excitation. The latter could be further tested by electrical stimulation of the olfactory nerves or the bulbs themselves. Tetanizing bulbs which had ceased activity restored spontaneous potentials in 3 of 5 cases. The potentials were feeble and irregular and resembled those usually seen some 20 min. prior to inactivity. When the nerves were stimulated (10 sec. 40 shocks per sec.) in the freshly isolated brain, the regular rhythm was disrupted and replaced by irregular small 30 to 50 per sec. waves. This picture outlasted the stimulation for 2 to 3 sec., when the 6 per sec. rhythm reappeared, to increase in amplitude and regularity during another 2 to 4 min. (Fig. 1B); by this time i.e., 4 to 7 min. after stimulation, the regular rhythm had attained an amplitude 25 per cent greater than before stimulation and the intensity then fell slowly, over an average period of 12 min., until it became normal. More prolonged stimulation caused more enduring post-stimulation changes. In only 2 of 12 experiments was tetanization of the nerve without effect, synaptic transmission perhaps having failed in the 2 cases before the test.

Temperature

The temperature of the isolated brain was varied between 8 and 35°C. but usually only between 14 and 30°C. At lower temperatures potentials gradually flattened to basal level; at higher temperatures, wave amplitude,

after brief but conspicuous increase, fell abruptly and irreversibly. In moderate temperature ranges, amplitude and especially frequency of the rhythm increased with temperature (Fig. 1C). When the temperature was brought to a new level the brain attained the new temperature, as shown by a thermocouple, in 4 to 5 min. The rhythm change lagged behind this and might require 4.5 to 6.5 min. to reach a new steady value (Table 2). In 16

Table 2. *Exp. 3/8/38. Bullfrog. Ether at 2:00 p.m. Brain exposed 2:15 p.m., isolated (to middle of cerebral hemisphere) 4:35 p.m. Readings start at 4:50 p.m.*

Temp. (Centigrade) around brain	Time (min.)	Brain temp.	Frequency (per sec.)	Q_{10} of frequency	Amplitude (μV.)	Q_{10} of amplitude
23°	0	22.9	7.0		60	
to	to			2.2		
29°	0.25			} 2.5		} 1.9
	1.0	24.4	7.8		70	
	1.75	26.0	9.0		75	
	2.5	26.4	9.5	3.6	80	
	3.25	26.9	10.1			
	4.0	26.9	10.1		75	
	4.5					
to	to			2.7		} 1.5
23.5°	4.75			} 2.5		
	6.0	24.3	7.8		60	
*	7.6	23.6	7.5		65	
	16.0	23.2	6.0		70	
to	to			1.5		
16°	16.25			} 2.1		
	17.0	20.2	5.5		65	
	17.3	19.0	5.1		55	} 2.1
	18.25	17.7	4.0	5.7	55	
	19.4	17.0	3.6		45	
	20.65	16.5	3.4		40	
	23.1	16.2	3.0		40	
to	to			3.7		} 2.7
23.3°	23.75			} 3.0		
	25.5	21.5	6.0		80	
	27.75	22.7	6.3		80	

* Q_{10} between 26.9°–16.2° is 3.1, but this high value must be discounted because of a break in the experiment at this point.

experiments in the moderate temperature range, the Q_{10} of frequency fell between 2.1 and 2.6, with an average of 2.3. The Q_{10} for amplitude, although of the same order, was more variable. This was due to greater dependence of amplitude than of frequency on the time of exposure to each temperature and to "spindling" of wave trains, which necessitated the arbitrary use of half the maximal amplitude for the calculations. A typical experiment is shown in Table 2, and four are plotted in Fig. 2 where the Q_{10} is calculated from Belehrádek's (1935) formula:

$$Q_{10} = \left(\frac{K_1}{K_2}\right)^{10/t_1 - t_2}$$

where K_1 and K_2 are the wave magnitudes at the Centrigrade temperatures, t_1 and t_2, respectively. The Q_{10} is greater in the lower temperature range (cf. Gasser, 1931), and the coefficient tends to be greater for a given temperature step on passing from the lower to the higher value than vice versa. Besides thermal control of frequency and amplitude, the regularity of the waves consistently improves, with a rise in temperature, while cooling al-
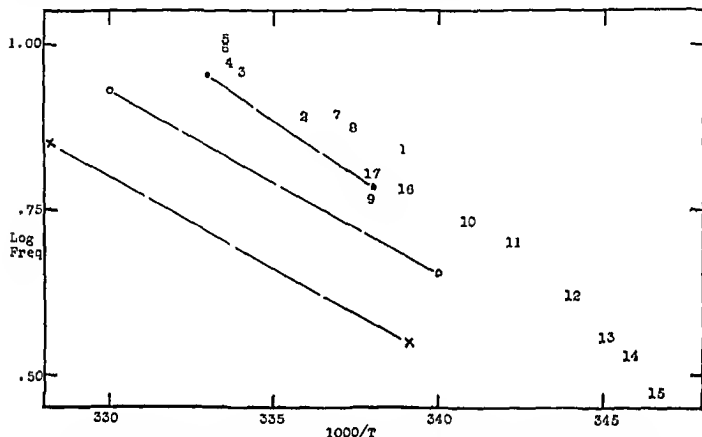

FIG. 2 Log. frequency plotted against reciprocal of absolute temperature. ×, 0 and • data from 3 separate brains. The numerals are the observations of Table 2, the number giving the order in which observations were made, plotted to these coordinates.

ways makes the wave shape more irregular (Fig. 1C). Greater regularity means a better alignment of activity in time, regardless of what components make up the wave. Consequently the process of synchronization is facilitated by a rise in temperature.

Osmotic pressure

Addition of sucrose or glucose to the Ringer's solution, sufficient to increase osmotic pressure by 50 per cent, does not affect potentials. Doubling the osmotic pressure with either sugar caused a change after 3 min. soaking in 4 of 9 experiments. The waves slowed 15 to 20 per cent, e.g., from 6.1 to 4.9 per sec., increased in regularity, and became 10 to 20 per cent larger (Fig. 1D a, b). The same osmotic change produced by doubling the Ringer salts, in 3 experiments, caused marked irregularity with the appearance of frequencies of 10 to 40 per sec. and a great loss of amplitude, not due solely to increased conductivity. Diluting Ringer's solution with an equal volume of water lowered the normal frequency only 10 per cent and made

the waves rather more regular; their amplitude was increased by half, after correcting for resistance changes. In water, potentials soon became irregular and disappeared after about 15 min. Most of the effects resulting from altered salt concentration depend more on specific ion changes than on changes in osmotic pressure.

H-ion concentration

Soaking in 0.013 M phosphate buffer has no effect on potentials. At pH values below 6.2 or above 7.8 changes are seen after 5 min. soaking. In acid solutions (acetate buffer) the 6 per sec. rhythm slows discontinuously to about 4 per sec. and the waves become less regular, with superposed frequencies of 15 to 40 per sec. (Fig. 1D c, d). In alkaline solutions (borate buffer), the normal rhythm became irregular and fairly strong waves at 15 to 30 per sec. are added (Fig. 1D e, f).

Electrolytes

The brain soaked in isosmolar (0.24 M) sucrose solution instead of Ringer's showed a progressive slowing of its rhythm and finally failed in 20 to 30 min. (Fig. 3A a, b, c, d, e). After 3 to 4 min. soaking, the 6 per sec. waves slowed sharply to 4 per sec. and increased 50 per cent in amplitude (e.g., from 120 to 180 μV.). After another 5 min., frequency fell to 3 per sec., and an increased inter-electrode resistance indicated fairly complete loss of salts from the brain's conducting fluids. After 12 to 15 min. total soaking, the frequency again dropped sharply, to 1 per sec., irregular waves at 15 to 20 per sec. were often superposed, and the amplitude became less. The negative limb of these slow waves was longer than the positive and the high-frequency discharges occurred on the slight plateau of greatest negativity. Further soaking usually caused gradual loss of these potentials. In one case (with K and Ca still present; see below) the 1 per sec. waves were again supplanted by extremely regular rhythms at 4 per sec. Diphasic spike-like potentials of 200 μV. interrupted this regular sequence, occurring first in groups of 3, then in mixed triple and single groups, then singly, and finally disappearing (Fig. 3B f, g, h). The diphasic spikes are probably produced by traveling rather than stationary potential waves.

These changes were only partially reversible. Soaking the brain in Ringer's solution, after activity had disappeared in the non-electrolyte solution, led to feeble irregular potentials. Restoration to saline at the slow wave stage replaced these with faster less intense ones which lack the normal regularity. A solution containing sucrose in place of NaCl, but with the other ions of Ringer's solution present in normal amounts, affected potentials as does pure sucrose, except that about twice as much time was required for each change and the slow waves did not become so extremely regular (Fig. 3B). Increasing the K-ion (to 0.004 M) partially antagonized the effects of sodium lack, and led to potentials much like those in Ringer's solution following sucrose (Fig. 3A f, g, h).

Sodium lack was mainly responsible for the changes seen in non-electrolyte solutions. Addition to or subtraction from Ringer's solution of NaCl
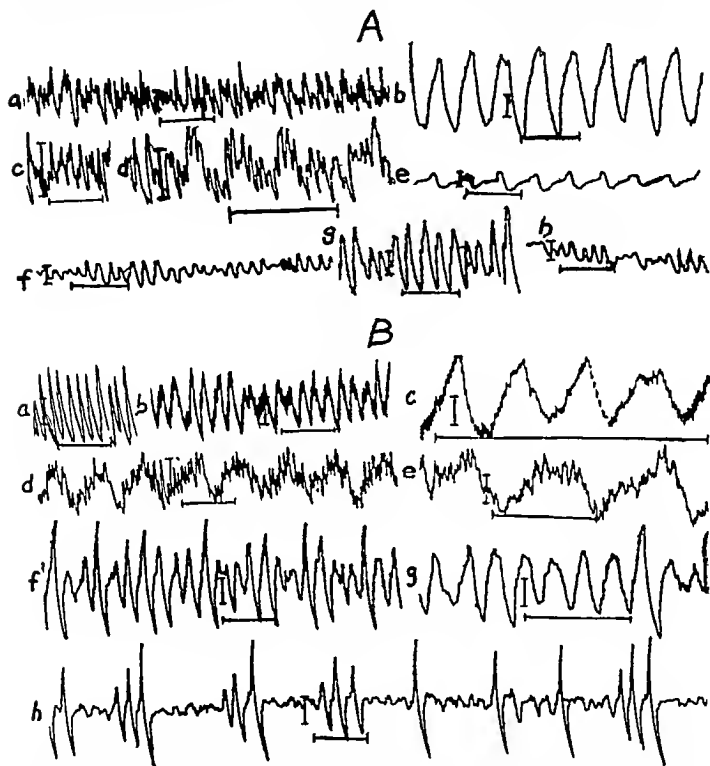

FIG 3 A a Normal in Ringer b after 4 min in 0 25 M sucrose c and d 3 min after return to Ringer e 8 min after replacing in sucrose f Another brain in Ringer's after 6 min in sucrose g 2 min after return to sucrose h 3 min after replacing sucrose with sucrose plus normal CaCl₂ and double KCl

B a Normal in Ringer b and c after 10 min in Ringer with sucrose replacing NaCl d and e after 20 min in same solution f and g after 30 min h 20 sec after g

in moderate concentration, *e g.*, 0.02 *M*, did not significantly affect potentials Doubling Na-ion produced feeble irregular rapid potentials, though the increased osmotic pressure, *per se,* would act in the opposite direction Replacing half the Na-ion of Ringer's solution with the isosmotic equivalent

of sucrose slowed the waves about 20 per cent after 3 min. soaking, 30 per cent after 10 min., increased their amplitude 30 to 40 per cent (*e.g.*, from 110 to 150 μV.), and increased regularity.

Potassium acted much like sodium, but much smaller quantities were effective. Isosmotic (0.125 *M*) KCl initiated rapid feeble waves which faded completely after soaking for 4 min. In 0.01 *M* concentration, this salt led to
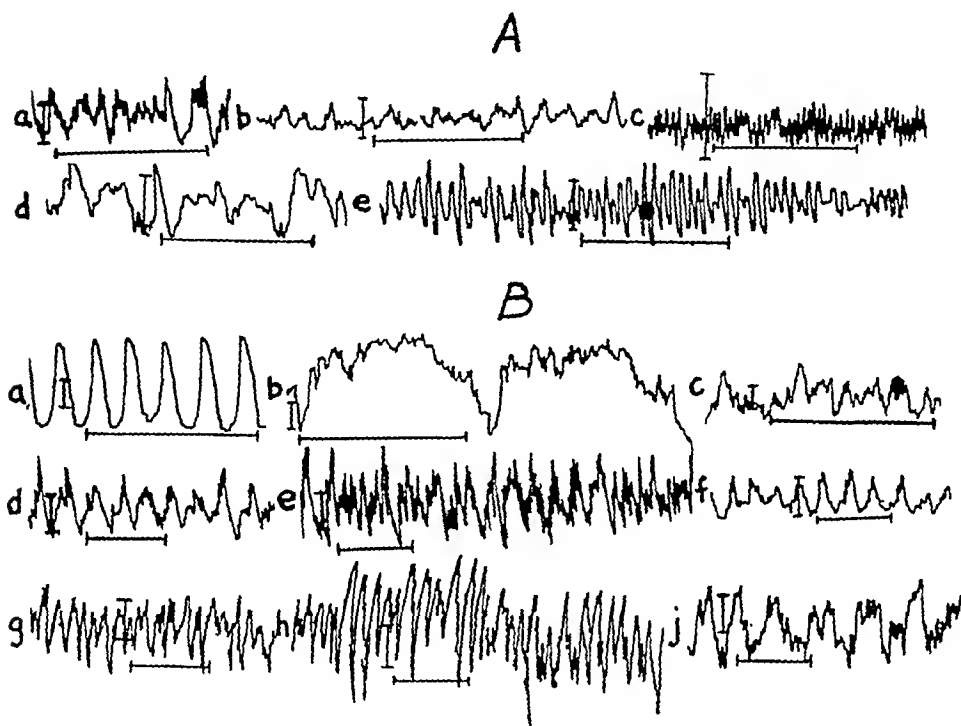
Fig. 4. A. a. normal in Ringer. b. after 3 min. in Ringer plus 0.003 *M* KCl. c. after 3 min. in Ringer plus 0.01 *M* KCl. d. another brain, normal. e. after 3 min. in Ringer plus 0.01 *M* KCl.

B. a. normal in Ringer. b. after 3 min. in Ringer plus 0.018 CaCl₂. c. 3 min. after return to Ringer. d. another brain, in Ringer plus 0.003 *M* CaCl₂. e. 3 min. after placing in Ringer plus 0.005 *M* Na citrate. f. 3 min. after return to Ringer plus 0.003 *M* CaCl₂. g. another brain, normal in Ringer. h. after 3 min. in Ringer plus 0.003 *M* MgCl₂. j. after 3 min. in Ringer plus 0.01 *M* MgCl₂.

irregular potentials in some cases, to regular waves at 20 to 30 per sec. in others (Fig. 4A). Concentrations between 0.003 and 0.01 *M* caused a sharp change from the normal to feeble somewhat irregular waves at 15 to 35 per sec. These changes were but little reversible when the brain was restored to Ringer. Weaker KCl, 0.002 *M*, however, gave a largely reversible increase in rate and decrease of amplitude (50 per cent) and regularity.

Calcium and magnesium (Fig. 4B) acted alike, and at similar concentrations, to change potentials in a manner opposite to that of sodium and potassium. Addition of 0.001 *M* CaCl₂ to Ringer's solution definitely slowed

the waves, and the effect was progressive with concentration to 0.007 M, which lowered frequency by 35 to 45 per cent (e.g., from 7 to 4 per sec.). Amplitude was not altered and regularity perhaps improved. Larger Ca-ion additions, 0.018 M, caused a discontinuous further slowing to 1 per sec. waves of the same amplitude with feeble irregular potentials at 10 to 20 per sec. superposed. Return to Ringer's solution restored more rapid waves, but they never regained the normal regularity. Removal of calcium affected potentials as did addition of potassium. Sodium *citrate* (0.004 M) in Ringer's solution produced irregular rapid waves, and the normal slow rhythm was again restored by Ringer's solution with added $CaCl_2$ (0.003 M) (Fig. 4B d, e, f). Sodium sulphate acted like citrate but greatly depressed amplitude as well (e.g., from 35 to 5 μV), and its action was irreversible.

Ion antagonism was apparent from the above results and clearly showed when potassium and calcium, especially, were balanced against one another. When both were removed from Ringer's solution, potentials in the remaining NaCl continued quite normal for about 10 min.; when both were added in equimolar amounts, up to at least 0.005 M addition, potentials also continued normal for some time; and when the excess of either ion had changed potentials, a slight imbalance in favor of the other improved and hastened the restoration toward normal.

Anions had little effect on potentials, except as they removed calcium. (Sulphate probably has a specific action as well.) At pH 7.4, mono- or dihydrogen phosphate (up to 0.013 M concentration) and bicarbonate (up to 0.02 M) were inactive. Substitution of half the chloride ion in Ringer's solution by iodide ion was without effect, and complete interchange of the two produced only minor differences. (Peculiar diphasic waves appeared at intervals in iodide.)

DISCUSSION

A single olfactory bulb can be made to vary its regular rhythm over a wide frequency range by appropriate change in environmental conditions. All rates have been encountered between 1 and 10 per sec., the values 13 and 17 appear and, less sharply, rates of 20 to 25, 35 to 40, and 45 to 55 (Fig. 5). Amplitude tends to decrease with increasing frequency, as does regularity, but large or small waves have been seen at all rates. Further, the shape of the individual regularly repeated waves can be deliberately changed from an approximate sine form to a variety of highly unsymmetric profiles (Fig. 5).

The relatively homogeneous cells of the bulb, probably only a few thousand separate units altogether, can thus manifest potentials of a fixed regularity and even a fixed amplitude over a fifty-fold range in frequency and a wide array of wave shapes. These facts speak strongly for the existence of individual neurone potentials of frequency and form identical with those of the recorded potential. This latter is the integral of the unit potentials and will reproduce them, except in amplitude, only if all units are similar and in synchrony. With asynchrony of units no regular pattern could be recorded unless each cell became active in a constantly repeated time se-

quence, as in the case of the flashing lights of a sign which are engaged in order by a rotating contact drum. The reverberating circuits postulated for the brain (Lorente de Nó, 1938) might afford just the neural mechanism required for such a delicate timing; but they are unfortunately ruled out by experiments with nicotine (Libet and Gerard, 1938; Schweitzer and Wright,
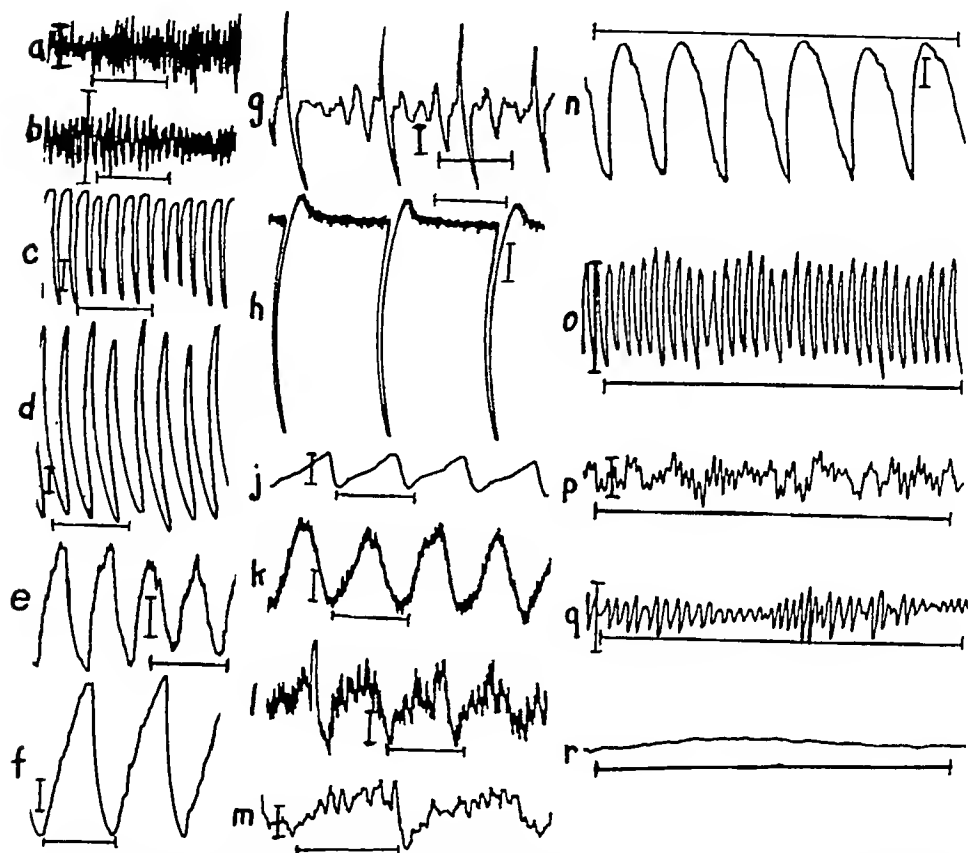

Fig. 5. Samples of rhythms obtained from isolated olfactory bulbs. a. freshly isolated, aberrant. b. 2 hr. in serum. c. freshly isolated. d. 3 min. in nicotine. e. 3 min. in sucrose. f. 6 min. in nicotine. g. 30 min. in Ringer with sucrose in place of NaCl. h. 3 hr. in stale serum. j. 10 min. in nicotine. k. 4 min. in nicotine. l. 15 min. in Ringer with sucrose in place of NaCl. m. 20 min. in Ringer with sucrose in place of NaCl. n. freshly isolated. o. small isolated bit of bulb. p. 30 min. in Ringer, aberrant. q. single separated bulb. r. control on optic lobe.

1938), which blocks synaptic conduction but leaves perfectly regular odd-shaped waves.

The range of frequencies exhibited by cells of one or few anatomical types (Gerard and Young, 1937; Adrian, 1937) must, then, represent a range of frequencies for each unit (see Blake and Gerard, 1937) or a regular sequence of action of varying numbers of cell groups. To the extent that doubling of frequency is accompanied by halving of amplitude, such an

alternation would be possible. But, though this is common, there are plenty of instances of a frequency increase with constant amplitude. Further, frequency may change smoothly with no discontinuities, or with jumps to values that are far from simple multiples of the initial ones. And, finally, any such beating in fractional groups would not account for a change to skew wave-forms, as produced by sucrose, nicotine, etc. The simplest hypothesis again seems to be that many single units beat in synchrony and so give a magnified record of their individual identical frequencies. This also fits with the fact that regular waves are obtainable from small fragments of teased olfactory bulb. It may be also, as Adrian (1937) suggests, that synchronizing occurs best at certain cell frequencies.

Changes in rate and form and, within limits, in amplitude induced by applied agents in *regular* potential waves would, then, be interpreted in terms of the individual neurone beat. But changes in regularity also occur—from an increased variability of successive distinct waves, through "fuzzy" waves with superposed potentials, and irregular alternations, to highly random potential swings—and these are best interpreted in terms of the mechanism of synchronization. Slight cell asynchrony will cause more apparent irregularity in short fast waves than in long slow ones; yet increase in temperature, which accelerates the rhythm, also improves its regularity. This is an especially clear case for a synchronizing mechanism, independent of the individual cell, which increases in efficiency with temperature. We shall, therefore, interpret the action of experimental procedures on potentials in terms of the unit neurone beat and the degree of synchronization of units.

Each cell exhibits a potential wave with amplitude, wave-length and shape characteristic for the extant conditions. The relation between frequency and intensity is especially important since, by physical analogy, at constant energy (from cell metabolism) the two should vary inversely. A change of rate without a change in amplitude, still more a parallel increase or decrease in both together—as in the case of altered temperature—must, then, indicate a change in available metabolic energy. (With improving synchrony, of course, amplitude could increase in the total record and not in the unit. This can often be excluded.) On the other hand, increased frequency with decreased amplitude might represent a change in the "setting" of some "trip" mechanism, with no change in the original metabolic sequences. The first case above would correspond to a change in the tension of a metronome spring, the second to a shift in position of the ballast on the pointer. Frequency variation due to alterations in metabolic rate would almost surely be smoothly continuous and essentially reversible, as with temperature; but variation due to resetting of the trip mechanism (some membrane property?) might easily be discontinuous and imperfectly reversible, as with altered ionic environment. Many agents unquestionably have a mixed action; and we shall report in a later paper studies, of the influence on these potentials of a number of metabolic inhibitors, accelerators, and other effective drugs, that help to clarify the cellular mechanisms which determine the electrical beat.

Little can be said as yet about the mechanisms which integrate and synchronize the beats of individual cells, but that such exist is certain. The findings with nicotine, etc., show that, though the passage of nerve impulses along conducting paths may play an important role, this is not a necessary or indeed the most important means of coordination. It has been suggested (Gerard, Marshall and Saul, 1936) that steady potential fields and polarization might contribute to synchronization, and a small constant current does increase cat brain rhythms as if improving cell unison (Dubner and Gerard, 1939). This experiment has not yet been performed on the frog brain, but some similar evidence is available from waxing and waning of waves.

Regular wave trains do not wax and wane but as the single waves become less constant this spindling is likely to appear. An interpretation would be that the cells are locked together by a strong synchronizing mechanism to give regular beats and that as this control is lessened the cells are more easily desynchronized, with first some fuzziness in the recorded potentials and then the coming in and out of phase which produces amplitude beats. There are indications that a steady potential of the brain mass is more negative during the large waves at the belly of a spindle and less so when the feeble waves occur at the ends of one. A greater constant potential is, therefore, associated with greater cell unison. That regular waves document a strong action of the synchronizing mechanism is further shown by attempts to disrupt them. It is constantly found that an agent which disturbs a steady rhythm, for example K ion, is effective in lower intensity with less regular waves. A very smooth beat persists in KCl concentrations which break up a less constant one and is disrupted only by greater concentrations.

These effects are seen with the passage of time *in vitro* and it is likely that one factor in the disintegration of the potentials of an isolated brain is the gradual failure of the synchronizing mechanism. The effects of stimulation might be partly via an improvement of this; and the fact that further isolation of a small bit of brain from a larger inactive mass brings out again a potential beat could also be explained in terms of leading from a few synchronized units rather than from many desynchronized ones. Finally, the improved synchrony induced by rise in temperature and by certain stimuli and by nicotine, sucrose or calcium, and the diminished synchrony with time of isolation, cold, potassium, etc., show that the synchronizing mechanism is a real entity with measurable properties permitting its further analysis.

It remains to note some impressive parallels between the behavior of brain and nerve. Brain rhythms have been compared to the oscillating afterpotentials of nerve (Gerard, 1936; Gasser, 1937). In mammalian nerve (Lehmann, 1937a and 1937b; less completely in frog nerve, Graham, 1933) the after-potential rhythm is increased and the "wave-length" shortened by raised potassium or by lowered calcium or hydrogen ion. The reverse ion changes, although augmenting the first negative after-potential, lengthen the oscillation period and may completely depress the waves. These effects are entirely comparable to those produced in the frog's olfactory bulb (and

cat's thalamus Dubner and Gerard, 1939), although the ions act on the brain more rapidly and in lower concentration. (Compare Dusser de Barenne, McCulloch, and Nims, 1937, on the influence of pH on cortical potentials.) That the frog brain should be relatively insensitive to pH changes is not surprising in view of the fact that the pH of the frog's blood normally varies rather widely (see Rohde, 1920); also frog nerve metabolism is fairly insensitive to pH change (Gerard, 1930). With after-negativity of nerve goes heightened irritability and even spontaneous discharge (Gasser and Grundfest, 1936; Lehmann, 1937a); with positivity, depression. The same is true for grey tissue (Adrian, 1931 and 1937; Hughes and Gasser, 1934; Eccles, 1936; Heinbecker, 1936; Barron and Matthews, 1938); and, in both, potassium and calcium powerfully control potentials, activity, and after-discharge (Gerard and Magoun, 1936). Further, in both brain and nerve there are similar powerful cation antagonisms while anions are relatively unimportant. Spike height in nerve is reduced by excess of either potassium or calcium (Graham, 1933) and the same is true in brain cortex (Dubner and Gerard, 1939).

Finally, nerve after-potentials are minimal in the well-rested tissue and increase with repeated activity. Their persistence, amplitude and oscillations, following a single impulse, depend on previous excitation and contemporary physico-chemical environment. The brain potential oscillations similarly are influenced by past activity as well as current environment; stimulation enhances the wave for a considerable time afterward and the usual rhythm fades out over hours in the unstimulated isolated bulb but can be restored for many minutes by a brief barrage of impulses. A like interpretation seems to fit the sequence of potential changes of the human cortex in sleep (Blake and Gerard, 1937; Blake *et al.*, 1939). It is also not improbable that the increased frequency commonly observed when a bit of brain is separated from a larger piece is due to injury potentials at torn processes, etc. It will be desirable to compare the behavior of such brain rhythms with that of the demarcation potentials of nerve and brain with change in time, temperature, ion concentration and the like.

The action of ions on brain and nerve metabolism has been reviewed elsewhere (Gerard, 1932 and 1937) and here it need only be mentioned that for brain respiration, as for its potentials, sodium and potassium synergize in increasing it, with potassium far more active per mole, and calcium and magnesium depress it; while anions have little effect (*e.g.*, Dickens and Greville, 1935). Whether the persistence of a rhythm in a salt-free medium means that inorganic ions are not indispensable to the potential control or that these are incompletely removed even from the cell exterior, cannot be answered from these results. At least the brain neurones have a sufficient store of substrate, and obtain adequate oxygen for the oxidative portion of their metabolism, to maintain their beat in isolation. Their ultimate failure is due only in part to loss of background excitation and must finally depend

on depletion of reserves or on disintegration of organization in the abnormal *in vitro* environment.

SUMMARY

The olfactory bulb of the isolated frog brain, which continues its *in vivo* electrical activity (especially a large regular 6 per sec. rhythm), was used to investigate physico-chemical and nervous factors controlling such spontaneous potentials.

Wave size and regularity are enhanced immediately upon isolation, without change in frequency, then gradually decrease to zero during about 3 hr. in Ringer's solution and 4 in serum. Wave frequency usually increases with each step of further isolation (single bulb, bit of bulb), and regularity, if anything, is improved.

Electrical stimulation can restore some activity of the "run down" brain; and, even in the freshly isolated one, stimulation of the olfactory nerve for seconds increases bulb potentials by 25 per cent for 10 min. following.

Rise in temperature, between 5 and 30°C., increases frequency (the average Q_{10} is 2.3) and amplitude, and always improves regularity. Cooling has the reverse effect.

Doubled osmotic pressure, radically reduced Na-ion, moderately increased Ca- or Mg-ions, or lowered pH produce slow waves; while increased K, Na, or pH and reduced Ca produce fast ones. Na and K are antagonistic to Ca, K in small concentrations being more effective than Na. Effects are generally progressive with concentration. Changes in frequency are usually discontinuous, and when extreme are irreversible. Anions are generally without marked effects.

Although originating in a small homogeneous neurone population, potential patterns may vary greatly in frequency, wave shape, and regularity depending on the factors studied. These and other related facts are discussed in relation to the problems of frequency and amplitude of single neurone rhythms and of the mechanism coordinating them to give the recorded potential. Significant parallels appear between rhythmic nerve potentials and those of cerebral neurones.

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BRAIN WAVE FREQUENCIES AND CELLULAR METABOLISM. EFFECTS OF DINITROPHENOL*

HUDSON HOAGLAND, MORTON A. RUBIN, AND D. EWEN CAMERON

From the Biological Laboratories, Clark University, and The Research Service, Worcester State Hospital, Worcester, Mass.

A VARIETY OF EXPERIMENTS indicate that the relative *frequency* of brain potentials, other things being equal, depends on the rate of respiration of the cells producing the rhythm. The temperature characteristics of alpha frequencies in persons subjected to diathermy coincide quantitatively with the major modal values encountered for cellular respiration *in vitro* (Hoagland, 1936a). Lowering of blood sugar by insulin, which results in subsequent lowering of brain sugar, causes alpha frequencies to diminish in rate, but with a lag. Injection of sugar restores the frequencies to normal (Hoagland, Rubin and Cameron, 1937; confirmed by Himwich *et al.*, unpublished). Sugar is probably the only fuel used by the brain (Himwich and Nahum, 1932). Thyroxin accelerates alpha frequencies (Rubin, Cohen and Hoagland, 1937), and nembutal, known to inhibit cellular respiration (cf. Page, 1937), slows the rhythms (Hoagland *et al.*, 1939). Himwich, Hadidian and Fazekas (unpublished) have demonstrated a relation between O_2 consumption of the brain and alpha frequencies, and Hoagland (1936b) has discussed physical models which would produce electrical rhythms at frequencies proportional to continuous chemical events of the type under consideration.

In view of these findings one might expect that dinitrophenol (an effective metabolic stimulant in respiring cell systems *in vitro*, as well as in intact mammals; cf. Dodds, 1934) should produce acceleration of brain wave frequencies.

METHODS

Electroencephalograms have been recorded on paper tape by two ink-writing undulators and matched amplifiers built by Albert Grass. The electrodes were wires attached to lead pellets 2 to 3 mm. in diameter, the pellets making contact with the scalp through electrode paste and held in position by collodion. One grid electrode was placed over the occiput, 2 cm. above the inion and the other was placed over the vertex. The indifferent lead was attached to the skin behind each ear and parallel leads from these attachments were brought together to form a common connection. Tests showed this ground lead to be electrically inactive. Our tape speed was 30 mm. per sec., our amplification 10 μV. = 1.5 mm., and our time constant 0.2 sec. Four persons were selected who showed dominant, clear, alpha rhythms at *both* occiput and vertex. These subjects were male schizophrenic patients who were more conveniently available for our tests than normal persons would be who might show equally dominant and, therefore, countable alpha waves at both vertex and occiput. There was nothing about the electroencephalograms of these patients that would characterize them as abnormal. As in a number of persons we have examined, the

alpha frequency from the vertex was uniformly about one cycle per sec. slower than that from the occiput. The simultaneously paired records showed electrical independence of each other, as would be expected (cf. Rubin, 1938).

Records were made on the subjects while they were reclining with closed eyes. On the first experimental day, prior to medication, about 5 min. of continuous records were taken, after which, at 1:30 p.m., each subject was given by mouth doses of 4.0 mg. per kg. body weight of 2, 4 dinitrophenol, and at intervals throughout the afternoon other 5-minute samples of electroencephalograms were made. On each of the 4 following days the same dose of dinitrophenol was administered at 1·30 and records were obtained 2 hr. later. The clear alpha frequencies were counted from 100 sec. samples (*ca.* 1000 alpha waves per sample) and their mean frequencies per sec. determined for purposes of plotting.

RESULTS

Figures 1 and 2 show the results obtained with two of the subjects which, in general, are typical of the four. Frequencies from both vertex (lower
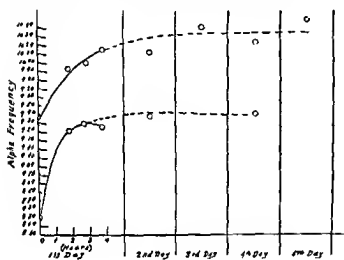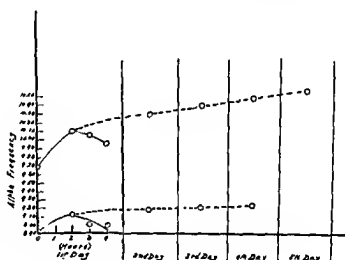
FIG. 1 (see text)   FIG. 2 (see text)

curves) and occiput (upper curves) are accelerated the first 2 or 3 hr. on the first day. Later the dinitrophenol effect apparently wears off and the frequencies tend to fall (solid curves). However, some residual effect remains since the same dose given at 1:30 on subsequent days gives for readings 2 hr. later a steady, slow rise in frequencies (dashed curves). On the 5th day alpha waves from the vertex in these 2 cases were irregular and no longer countable, though occipital alphas were as dominant as ever. The alpha waves from the vextex in the other 2 patients remained clear on the 5th day but the data throughout were somewhat more variable. Hoagland (1936a) showed that the probable errors of alpha frequencies counted in this way involving comparable data are of the order of ± 0.05 cycles per sec. This is about the diameter of the circles making up the plotted points. The curves are, therefore, highly reliable statistically. Data obtained 4 days after the termination of dinitrophenol medication showed that all of the frequencies were still abnormally high. A week later, however, they were back to the normal preexperimental level.

These experiments make it clear that dinitrophenol accelerates cortical brain wave frequencies in a way to be expected of this specific respiratory

stimulant, if our premise at the beginning of this paper is correct. It is especially interesting that rhythms from both occiput and vertex are independently accelerated in each person to approximately the same degree and along similar curves. Individual differences of the subjects are also suggestive.

Summary

1. Occipital alpha brain wave frequencies and occipitally independent alpha type waves from the vertex, which are about one cycle per sec. slower, were studied simultaneously with independent double recording systems in 4 subjects before and after doses of dinitrophenol.

2. The independent rhythms from both regions are accelerated along two smooth curves in a manner to conform with the view that the frequencies, under the conditions of these experiments, are a measure of cortical respiration.

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THE CONDITIONED REFLEX OF BLINKING

G. MARTINO

Istituto di Fisiologia Umana R. Università di Messina, Sicily

FOR SEVERAL years work has been carried on in this laboratory on a particular conditioned motor reflex whose elaboration it has been possible to obtain in the dog—the reflex of blinking. The observations so far made have been published in various Italian journals, and since they allow of certain conclusions of a general nature, particularly in regard to the intimate mechanism of conditioned reactions, it is desirable to describe them here.

The unconditioned (innate or original) reflex of blinking, in the dog, consists in a rapid contraction of m. orbicularis oculi in response to the action of an adequate stimulus on a reflexogenous cutaneous area (in the homolateral half of the face). It is under the control of a cortical motor centre of the gyrus sigmoideus (whose activity is of a reflex nature), and a state of activity in this cortical centre, made manifest by the contraction of the orbicularis oculi on the opposite side, is provoked by centripetal impulses which start from this well-defined reflexogenous cutaneous area. Electric stimulation of this area invariably provokes in all dogs the reflex contraction of the orbicularis oculi, and frequently repeated association of indifferent sensory stimuli (of various kinds) with the innate reflex has permitted the development of *conditioned reflexes of blinking.*

The analysis of these has given interesting results on the problem of the intimate mechanism of associative reactions. According to the concept of Pavlov, the development of conditioned reflexes is attained by substitution in the arc of the congenital reflex of the unconditioned afferent path with a new (conditioned) centripetal path. Only the last segment of the arc would remain unchanged—the part which conveys the impulses to the peripheral effector organ. We found that after stovainisation of the reflexogenous cutaneous area of the congenital arc, the conditioned stimuli—luminous[1] or auditory[2]—lost their previously acquired capacity of exciting the reaction of blinking. The reflexogenous area of the unconditioned arc is therefore an essential part in the mechanism of the conditioned reflex; in other words, the persistence and integrity of the congenital afferent path represents an *essential condition* for the manifestation of associative reactions. It is not then by the replacement of the common centripetal path by the conditioned afferent one that "action at a distance" (*Fernwirkung*) of conditional exciters becomes possible.

We thought that this action might consist in the facilitation (*Bahnung*) of the congenital reflex. In this case the capacity of the conditioned stimulus to provoke the active state of the reflex centre would be only apparent. In reality that stimulus would only cause an increase in the excitability of the centre, and the active state, in the last analysis, would always be induced, in the associative reaction as in the congenital, by unconditioned afferent stimuli.

In order to test the validity of this hypothesis we studied the activity of

the motor centre during the action of conditioned stimuli in dogs trained to conditioned blinking reflexes.[3] We found an increase in its faradic excitability and observed, after central treatment with strychnine, that provocations of reflex clonus and of reflex epileptic forms by peripheral stimuli were facilitated.*

Another proof of the effect of the conditioned stimuli on the excitability of the reflex centre is furnished by the following observation: during the elaboration of an associative reflex the threshold of the unconditioned stimulus is lowered progressively as the efficacy of the conditioned signals progressively increases. Thus an unconditioned stimulus, insufficient in itself to provoke the reflex contraction of the m. orbicularis oculi, becomes capable of doing so if preceded by a conditioned signal, even though the latter by itself is insufficient to cause the reaction of blinking.[4]

Finally, a further (indirect) demonstration of these effects of the sensory associative stimulus on the excitability of the reflex centre is found in the following experimental observations: In this Institute it had been shown previously that a functional connection exists between a given point of the occipital cortex (visual area) of the dog and the motor centre of the m. orbicularis oculi of the same hemisphere, so that the reflex activity of the latter centre is facilitated by direct circumscribed strychninisation of a point in the occipital cortex.[5] Two distinct conditioned reflexes of blinking were then elaborated in a dog in response to luminous stimuli—with the left eye to red light, and the right to violet. After the application of strychnine to the occipital cortex of the left hemisphere violet light showed the power of provoking clonus of the right m. orbicularis oculi followed by generalized attacks of epileptic nature (arising from this muscle). On the other hand no effect was obtained by stimulation with red light. After strychninisation of the occipital cortex of the right hemisphere we obtained on the contrary clonic twitches of the left orbicularis—and subsequent epileptic attacks—under the action of red light but not under that of violet.[6]

This therefore is the effective mechanism of the associative reactions: the conditioned impressions from the sensory cortex bombard the motor centre and augment its excitability while its active state is still induced by the centripetal impulses congenitally destined to this purpose, that is, by those which start from the reflexogenous region of the unconditioned arc. "It cannot be denied, indeed it is necessary to recognize, that centripetal impulses are continually starting from the peripheral reflexogenous zones in response to the influence of numerous stimuli. Such impulses are normally inactive and therefore in ordinary circumstances the reaction—motor or secretory—does not take place in the absence of the adequate stimulus, natural or artificial, but this is dependent on the degree of excitability of the reflex centre. If this central excitability is increased beyond certain limits, then these subliminal af-

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* Diminution of chronaxie—i.e., increase of excitability—in the reflex centre under the action of conditioned stimuli has been observed by Chauchard and Drabovitch (C. R. Soc. Biol., Paris, 1936, 2: 67).

ferent impulses, usually inactive, may prove themselves sufficient to excite the activity of the reflex centre."[7]

On account of the symmetrical bilateral organization of the cortical centres the reflex of blinking makes possible a rigorous comparison between the effects of different conditioned stimuli.[8] In the same animal it is, in fact, possible to obtain the simultaneous elaboration of *two different* conditioned reflexes of blinking. This, obviously, is not possible for the reflex of the salivary secretion. The comparison between the effects produced by different stimuli on different animals, or even on the same animal at different times, cannot furnish data of much value because of the importance of the individuality of the animal and of numerous other factors in the elaboration and extinction of associative reflexes.*

In agreement with the observations of Pavlov on the conditioned reflexes of salivary secretion we have found that luminous stimuli are less effective than sonorous in the elaboration of the associative reflex. Moreover the reflexes elaborated by means of sonorous signals have shown a greater resistance to the phenomenon of extinction.

In other experiments carried out on a dog trained to a double conditioned reflex of blinking by luminous signals (violet light for one eye, red light for the other) it was possible to recognize certain effects, related to the *quality* of the luminous conditioned stimulus, on the processes of facilitation and inhibition. In particular, red light ($\lambda = 7200 - 6000$ Å) showed itself less effective than violet ($\lambda = 5500 - 4250$ Å) in the elaboration of the associative reflex, and the reflex was less resistant to extinction in the former than in the latter case. Since the greater or less effectiveness of the conditioned agent depends on the greater or less sensitiveness of the sensory analyser (Pavlov[9]) this result shows that the optical analyser of the dog is more sensitive to rays of wave length corresponding to our violet light than to those of wave length corresponding to our red light. This behavior is similar to that of the optical analyser in man.[10] Violet light showed itself more effective than red in restoring an associative reaction which had been extinguished. Moreover in the case of red light the impulses showed a greater tendency to irradiation; in that of violet light, to concentration. Pavlov considered that the tendency of impulses to irradiate or to concentrate varies according to the energy of the sensory agent; stronger impulses tend more readily to concentration, while the weaker are generally more disposed to irradiation. Our observation therefore constitutes a further

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* Pavlov wrote concerning the phenomenon of "internal inhibition (extinction)": "It is necessary first of all to mention the influence of the individuality of the animals. In some, other things being equal, the conditioned reflexes are rapidly extinguished, in others very slowly. This fact is closely connected with the general character of the nervous system of the animal. In nervous and excitable dogs the reflexes are usually slowly extinguished; in quiet and calm dogs they are extinguished rapidly. The degree of development of the reflex also plays its part. The younger and the weaker it is, the more rapidly it is extinguished, and vice-versa. The intensity of the absolute reflex with whose aid the conditioned has been formed exercises a well-defined influence on the rapidity of extinction . . . " (*Leçons sur l'activité du cortex cérébral.* A Legrand. Paris, 1929, p. 52).

proof of the difference of energy of the two luminous stimuli of different quality.

We have also obtained the contemporaneous elaboration of two conditioned reflexes by means of sound signals of different quality:[4] blinking of the left eye to the note mi[7] (5520 vibrations) and of the right to the note fa[7] (5568 vibrations). Although the interval between the two notes is hardly a semitone, the dog quickly learned to distinguish between the two signals so that in a short time absolutely correct conditioned responses were obtained. The note mi[7] showed itself more effective than fa[7] in the elaboration of this conditioned reflex and the reflex produced was more resistant to extinction.

In the course of the experiment we observed on several occasions typical phenomena of external inhibition in which the established associative reflex became weaker, or even completely vanished, as a result of noises accidentally penetrating into the laboratory. In such cases we observed sometimes a marked diminution in the *power of discrimination*, so that the dog responded, for example, to the signal mi[7] by a contraction of the right orbicularis (instead of the left) or to fa[7] by the left orbicularis (instead of the right). On some rare occasions a similar diminution of discriminative power was observed as a result of fatigue, at the end of an unduly prolonged trial. These are inhibitory effects, which may logically be attributed to a relaxing of attention. Other observations on the processes of inhibition, or disinhibition, etc., are in agreement with Pavlov's results on the associative reflexes of salivary secretion.

It is appropriate to mention here the recent interesting experiments of Gantt and his collaborators on the anatomical structures engaged in the elaboration of the conditioned reflex. They have shown that in the elaboration of an associative reflex the external conditioned stimulus may be replaced by artificial stimulation (faradic) of various parts of the central nervous system.[11] This constitutes an indirect confirmation of my views on the intimate mechanism of conditioned reactions: a proof that no necessity exists for a special arc of the associative reflex substantially different from the congenital one. We could not otherwise explain the effects of the so-called "intra-neural conditioning" (Gantt) except by invoking the phenomenon of *Bahnung*. In no other way save by increase in the excitability of the reflex centre could the artificial stimulation of the cerebellum or of the cerebral cortex or of the posterior spinal roots elicit the reaction which depends upon that centre. Analogous processes of facilitation ("sekundäre Bahnung" of the German investigators) have long been known. A clear distinction between the factors which determine the active state of the reflex centre and those which increase its excitability is therefore an indispensable condition for a correct understanding of the mechanism of associative reflexes.

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SUR UN PHÉNOMENE DE FACILITATION RÉTROACTIVE DANS L'EXCITATION ÉLECTRIQUE DE BRANCHES NERVEUSES CUTANÉES (SENSIBILITÉ TACTILE)

H. PIÉRON et J. SEGAL
Collège de France, Paris, France

INTRODUCTION

AU COURS de recherches sur les processus d'excitation de la sensibilité tactile, par stimulation électrique, au moyen de décharges de condensateurs, d'une branche cutanée sensitive (un rameau de la branche latérale cutanée externe du médian, à la base de la première phalange), certains faits d'allure paradoxale ont été mis en évidence, faits signalés dans la note préliminaire que nous avons publiée sur ces recherches.[1] Ils consistent essentiellement en un accroissement de l'efficacité d'un choc sous-liminaire sous l'influence d'un choc consécutif, dans une assez grande marge d'intervalles, et en un raccourcissement du temps de réaction à une stimulation par un choc, du fait de la production consécutive d'un second choc, dans les limites d'une marge d'étendue analogue.

Ces faits posent un problème dont la solution doit impliquer certaines conséquences au point de vue des processus de réception sensorielle et de leur mécanisme cérébral. Nous indiquerons tout d'abord la méthode et la technique des recherches, puis les données générales obtenues, avant de décrire les phénomènes de facilitation rétroactive observés et d'en discuter l'interprétation.

I. METHODE ET TECHNIQUE EXPERIMENTALE

Après divers tâtonnements relatifs à des stimulations électriques de plusieurs branches nerveuses, nous avons choisi un rameau terminal uniquement sensitif et accessible dans une région où l'on ne risque plus d'exciter des muscles et de provoquer des contractions avec leurs répercussions sensitives.

A la base de la première phalange du médius, en tâtonnant latéralement avec une électrode de Bourguignon, on arrive à déterminer un point où les stimulations d'un rameau superficiel, sur le territoire du médian, provoquent des sensations de choc tactile au niveau de la 2 ème phalange dans une région latérale, près de l'articulation proximale. Avec des décharges de condensateur assez brèves, on ne provoque d'impression douloureuse que sous des voltages assez élevés, au triple environ du voltage liminaire quand la durée de décharge est égale à la chronaxie. Nous avons donc utilisé systématiquement des durées de décharge égales à la chronaxie, déterminée chaque fois au préalable, ce qui nous a permis d'obtenir des sensations exclusivement tactiles, pour une marge assez grande, relativement, des intensités de stimulation. En utilisant, ainsi, des décharges de condensateurs, il était possible de réaliser des stimulations multiples, de durée définie, en réglant leur nombre et leur intervalle grâce à un dispositif contacteur constitué par un cylindre tournant.

Pour réaliser les voltages élevés nécessaires, atteignant plusieurs centaines de volts, nous avons dû nous servir de piles sèches avec réglage direct des tensions par le jeu des fiches (échelons de 1.5 volt). Un potentiomètre à faible résistance aurait vite mis les piles hors service et une grande résistance aurait rendu trop lente la charge des condensateurs qui devait être très rapidement effectuée. Toutefois un potentiomètre de faible résistance a été quelquefois utilisé pour une stimulation additive dans la mesure des seuils différentiels. Le dispositif de stimulation est conforme au schéma classique.

Circuit de décharge Le condensateur variable *C* (de la figure 1) est branché successivement sur la pile P_1 et sur le circuit de décharge, qui comporte une résistance de 25,000 ohms en série avec le sujet, une autre de 7,000 ohms en parallèle avec lui, et une troisième de 18,000 ohms en série avec le condensateur Dans ces conditions 0 001μF correspond à une durée de décharge d'environ 0 009 msec Pour déterminer la *rhéobase*, on court circuitait au moyen du manipulateur M_1 Avec le manipulateur M_2 on pouvait brancher à volonté dans le circuit une pile P_2 apportant un voltage supplémentaire déterminé, quand on faisait varier l'intensité de stimulation au cours des chocs consécutifs (et, dans certains cas, un potentiomètre à faible résistance pouvait être branché en P_2)

Relais de commande Normalement, les stimulations étaient assurées par des contacts au niveau du relais R_1 spécialement construit sur le type des signaux de Després, et capable de suivre des fréquences d'intermittences s'élevant jusqu'à 400 par seconde, grâce à sa très faible inertie, et à l'absence de fréquence propre, ce qui permettait, au moyen du contacteur, d'imposer des rythmes de stimulations brèves définis en nombre et en fréquence

Contacteur Le contacteur consistait en un cylindre métallique recouvert d'un papier calque
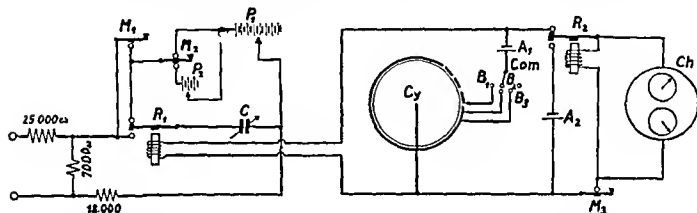
FIG 1 Schéma du dispositif utilisé pour la stimulation par décharges de condensateurs uniques ou répétées a intervalles variables, et pour la détermination des temps de réaction (signification des lettres et explication dans le texte)

dans lequel étaient découpées des ouvertures formant des séries linéaires parallèles, de nombre et d'intervalle déterminés

Un balai frottant sur la surface, et fermant un circuit, au passage de chaque ouverture d'une série au niveau de laquelle il était placé sur son support, permettait l'envoi des décharges de condensateurs Le circuit, commandant le relais, comportait un accumulateur A_1, le balai, la masse du cylindre, et le bobinage du relais La durée exacte du contact, assurant, par la manœuvre du relais, la décharge du condensateur, n'avait pas d'importance Dans l'intervalle de deux contacts, le condensateur était rechargé

En plaçant sur support, côte à côte, trois balais B_1, B_2, B_3, chacun devant une série de perforations du papier du cylindre, on pouvait, par le jeu du commutateur, Com, changer la série des stimulations, comparer par exemple 1 choc unique et 2 chocs, ou 2 chocs séparés par un certain intervalle et 2 chocs semblables séparés par un intervalle plus petit ou plus grand, etc

Dispositif des temps de réaction Pour la mesure des temps de réaction, le balai, en réalisant le contact par l'ouverture du papier, commandait, outre le relais R_1 à retour spontané (commandé par un ressort), un relais R_2 à déclenchement spontané irréversible, et branchant un chronoscope de Hipp sur le circuit de l'accumulateur A_2, la marche de l'aiguille, dont le départ était déclenché dès la commande du relais par l'accumulateur A_1 avant la constitution du circuit de A_2, était assurée jusqu'à ce que le sujet réalisât l'arrêt, par sa réaction, en interrompant le circuit par jeu du manipulateur M_3 (pressant sur le bouton d'une clef de Morse), provoquant en outre le retour du relais R. en position de départ et prêt ainsi pour une nouvelle mesure de temps de réaction

Électrodes Les électrodes, du type impolarisable zinc—sulfate de zinc, comportaient une anode indifférente constituée par une large plaque de zinc entourant le poignet avec, interposé, du coton imbibé de la solution de sulfate de zinc, une bande élastique maintenant la plaque, et une cathode active, spécialement construite pour l'application sur le doigt (médius gauche, face externe), celle ci était faite d'un petit bloc cylindrique d'ébonite avec noyau de zinc, et, dans une cavité rejoignant le noyau, un bouchon de coton imbibé, la

base du bloc, assez large, épousant la forme du doigt; mais la partie active de l'électrode restait limitée à une surface de petit diamètre (environ 3 mm.). Avec fixation par un élastique à tenseur, une telle électrode reste bien en place même au cours des mouvements du doigt, et, grâce à l'étanchéité, le desséchement est très lent, et l'on peut opérer pendant plus d'une heure sans toucher à l'électrode.

Le sujet était placé dans une pièce, l'expérimentateur dans une autre (en sorte que les manipulations n'étaient point vues et que le déclic du chronoscope n'était pas entendu). Une signalisation lumineuse permettait les communications entre l'expérimentateur et le sujet.

II. Données Generales sur les Faits Observés

Une décharge très brève de condensateur engendre une sensation tactile de choc dont on a toutes raisons de penser qu'elle correspond à l'envoi d'une influx unique, sans qu'il y ait nécessité d'une itération. Dès lors la mesure d'une chronaxie sensitive véritable peut être envisagée comme correcte. Il y a probabilité en effet que, dans la détermination de la rhéobase, donnant bien au seuil une sensation tactile de fermeture, ce sont les mêmes fibres qui sont mises en jeu.

Avec 8 sujets et 28 déterminations, la valeur moyenne de la chronaxie a été de 0,38 msec. Mais, avec des décharges répétées, on a un abaissement du seuil. Le voltage liminaire, très légèrement abaissé (de l'ordre de 1 per cent) pour un double choc avec un intervalle de 0,80 sec. est diminué de près de 10 pour cent lorsque l'intervalle des deux chocs est de l'ordre des centièmes de seconde. Le voltage liminaire, pour des stimulations intermittentes continues (au rythme de 25 à la seconde) est abaissé d'environ 13 per cent, l'intégration portant sur 4 stimulations successives au moins. Avec l'augmentation du voltage (en durée fixe) l'intensité du choc s'accroît, et la sensibilité différentielle est fine (pouvant atteindre, comme optimum, moins de 1 per cent chez certains sujets). La variation du seuil différentiel relatif avec le niveau d'intensité a une allure assez complexe.

Pour des stimulations rythmées, on a une sensation de type vibratoire, un "flicker" tactile, avec tendance à la fusion vers 200 par seconde pour des intensités faibles, proches du seuil. Aux intensités un peu élevées, la stimulation intermittente met en jeu beaucoup plus tôt la sensibilité algique que les stimulations isolées, d'où un picotement qui rend difficile la détermination de la fréquence critique de fusion tactile. La sensibilité différentielle à la fréquence, pour une stimulation intermittente, dans ces conditions, s'est montrée assez grossière, des variations, pour être perçues (autour d'une fréquence de 50 à la seconde) devant atteindre un taux de 30 à 50 per cent. Dans la stimulation intermittente prolongée. l'adaptation se produit, avec extinction totale d'autant plus précoce que le niveau d'intensité est plus las, 5 secondes aux environs du seuil, 35 secondes pour un voltage 1,25 fois supérieur. Ce temps d'adaptation diminue progressivement quand on reprend, après un court repos, les stimulations rythmées au même niveau d'intensité.

III. La Facilitation Rétroactive Par Double Choc

Au cours de l'étude des processus de sommation, des séries de déterminations furent faites, chez divers sujets, du voltage liminaire par application

d'un choc unique de durée chronaxique, ou de deux chocs consécutifs, de cette même durée, séparés par un intervalle variable, l'efficacité de la sommation se manifestant encore pour un intervalle de 0,4 sec., mais non pour un intervalle de 0,8 chez la plupart des sujets.

On cherchait alternativement, à plusieurs reprises, le seuil du choc unique et du double choc. Voici, par exemple, des valeurs obtenues chez un sujet, avec un intervalle de 20 msec. (durée chronaxique de décharge de 0,48 msec.):

Nombre de chocs:	1	2	1	2	1	2	1
Voltage liminaire:	129	102	126	88	124,5	99	123

Avec un intervalle de 166 msec., on obtient chez le même sujet:

Nombre de chocs:	1	2	1	2	1
Voltage liminaire:	135	126	133,5	124,5	130,5

Or, en appliquant le double choc avec le voltage correspondant au seuil du choc unique, le sujet, pour cet intervalle de 166 msec., percevait nettement les deux chocs consécutifs, mais, en abaissant le voltage, continuait à percevoir les deux chocs et cela jusqu'au voltage liminaire. Ainsi à 126 volts, en appliquant les deux chocs de condensateurs consécutifs, il y a perception de deux chocs mécaniques; avec ce voltage, le choc électrique unique est inefficace et ne donne pas naissance à une sensation; en augmentant le voltage jusqu'à 135 volts par unités d'1, 5 v., on obtient chaque fois, avec ce double choc électrique, la perception de deux chocs mécaniques mais avec un seul choc électrique, aucune sensation, tant qu'on n'a pas atteint 133,5 volts,—le choc unique commençant alors à être efficace (au lieu de 135 v., préalablement). Pour des intervalles plus courts, les deux chocs mécaniques étaient aussi perçus avec des voltages inférieurs au seuil d'efficacité du choc électrique unique, lorsque les deux décharges de condensateur se suivaient, et l'appréciation comparative de l'intervalle des deux chocs était correcte, les deux chocs paraissant bien plus rapprochés pour un intervalle plus court des deux décharges.

Ainsi la sommation ne se traduit pas seulement par une efficacité plus grande de la deuxième décharge capable de franchir le seuil pour un voltage infraliminaire en cas de décharge unique, mais par un accroissement d'efficacité de la première décharge elle même, ce qui comporte un processus de *facilitation rétroactive*. Le phénomène s'est montré général chez tous les sujets pour des intervalles convenables.

Toutefois, chez un seul des sujets la perception du double choc mécanique persistait de façon constante jusqu'au seuil d'efficacité de la double décharge; chez les autres sujets, en général, au seuil de la double décharge, l'impression, qualitativement différente de celle du choc unique, ne comportait pas toutefois une perception distincte et nette des deux chocs mécaniques consécutifs. Mais, dès que le potentiel liminaire était accru de 1,5 volt, cette perception distincte se produisait toujours. Quand l'intervalle est seulement de 10 à 40 msec., il y a aussi une impression qualitativement différente de celle du choc unique, sans distinction nette de deux chocs, pour les voltages

intermédiaires entre le seuil du double choc et le seuil du choc unique. Nous allons donner quelques valeurs numériques chez trois sujets pour préciser le fait. Chez le sujet précédemment cité, voici des séries de mesures du voltage liminaire avec deux autres intervalles:

Nombre de chocs	1	2	1	2	1
Ecart de 83 msec.	124,5	108	127,5	112,5	127,5
——— 400 msec.	111	103,5	112,5	105	112,5

Dans tous ces cas, le sujet a perçu le double choc jusqu'au seuil.

Chez un autre sujet (chronaxie de 0,40 msec.) pour 83 msec. d'intervalle:

Nombre de chocs	2	1	2	2	1	2
Voltage liminaire	199,5	208,5	192	189	201	187,5

Le double choc est perçu déjà au début, à 199,5 v., et il l'est encore à 189 volts ensuite, le même voltage étant toujours inefficace avec une décharge unique.

Chez un troisième sujet, systématiquement, de nombreuses stimulations sont effectuées, avec décharge unique ou double, et la nature de la sensation de choc est indiquée chaque fois.

Avec 83 msec., d'intervalle (durée chronaxique d'excitation de 0,80 msec.), on obtient:

Nombre de chocs	1	2	1	2
Voltage liminaire	102	96	99	91

Avec 96 volts, puis avec 91 volts, déjà la sensation de double choc est accusée. Avec le même intervalle, le seuil avec décharge unique étant de 88 v., la double décharge donne une sensation à 80,5 v. Cette sensation n'est pas nettement de double choc, mais n'est pas non plus celle du choc unique, dès 82 v., le double choc est perçu nettement. Avec 166 msec. d'intervalle, le seuil pour la décharge unique est de 90 v., et pour la décharge double de 84 v.; à 85,5 v. la sensation de double choc est tout à fait nette; dans une autre série de mesures, le seuil pour la décharge unique est à 102 puis 103,5 v. A 97,5 v., avec double décharge, le double choc est perçu. Mêmes faits, chez ce sujet, avec des durées d'excitation, par décharge de condensateur, plus brèves (demi-chronaxie) ou plus longues (double chronaxie).

Voici donc le fait, d'allure éminemment paradoxale; une décharge inefficace, qui, si elle reste isolée, ne donne pas naissance à une sensation, devient efficace et provoque une sensation quand elle est suivie d'une autre décharge égale, et inégalement inefficace. Ce n'est pas seulement le première décharge qui, par un processus d'addition latente, rend efficace la seconde, mais c'est la seconde qui, par un processus de facilitation rétroactive, rend efficace la première.

IV. L'Accélération Réactionnelle Par Double Choc

La mesure des temps de réaction aux sensations de choc tactile provoquées par les décharges de condensateur de durée chronaxique indique une dé-

croissance très rapide de ces temps quand s'élève le voltage, à partir du seuil (marge réductible décroissant en fonction de la fonction quatrième de l'intensité de stimulation, en prenant pour unité d'intensité le voltage liminaire). Quand on stimule, par deux décharges consécutives de même intensité et de même durée, le temps de réaction est en général diminué; cette diminution est d'autant plus grande qu'on est plus près du seuil et que la marge réductible des temps est plus étendue. L'intervalle entre les deux décharges joue naturellement un rôle, l'action raccourcissante faisant défaut si l'intervalle est trop grand et faisant même place à une action allongeante près du seuil, mais se montrant maxima pour des intervalles d'une durée optima, sans que l'optimum ait été encore déterminé avec précision (l'optimum peut-être variable avec les sujets et aussi avec les niveaux d'intensité).

Pour comparer les temps de réaction avec un ou deux chocs électriques, on alternait la stimulation unique et la stimulation double. Voici, chez un sujet, entraîné et cohérent, des données relatives à ces valeurs des temps (en *msec.*), les intensités étant exprimées en multiples du voltage liminaire (avec choc unique) pris pour unité. Les deux valeurs indiquées à la suite représentent la moyenne arithmétique et le médian, qui est plus significatif, et la valeur entre parenthèses donne le nombre de mesures (Sujet P).

Intensité	Choc unique	Double choc à intervalle de				
		30 msec.	45 msec.	80 msec.	140 msec.	185 msec.
1	597–594(18)					
1,5	491–476(19)				475–472(18)	
	(349–346(19)				383–389(20)	
2	(325–329(15)	265–261(20)		293–303(15)		358–365(19)
	(309–309(17)	292–292(17)				
3	271–272,5(18)		267–268(19)			

Voici d'autre part quelques déterminations faites sur divers autres sujets.

Sujet	Intensité	Choc unique	Double choc à intervalle de			
			13–14 msec.	24 msec.	30 msec.	40–45 msec.*
H	1	259–230(19)	242–236,5(20)			
H	1,05	(185–183(13)	178–180,5(10)			
		(200–206(13)				197–194(15)
S	1,25	(308–294(14)		274–270(13)		
		(310–319(8)	286–280(12)			299–310(8)
H	1,44	175–176,5(22)	176–177,5(22)			
B*	2	(236–230(10)	223–222(7)		232–231(9)	
		(364–360,5(10)				

*À 80 msec. le sujet B avec l'intensité 2 donne 285–278(11), et à 140 msec. il donne 312–295(9).

L'action raccourcissante augmente-t-elle par action de plusieurs décharges consécutives? Cela paraît se dégager de quelques données, à condition que

l'intervalle entre les décharges consécutives *ne soit pas trop long*. Voici par exemple une série de mesures réalisées chez le sujet P avec des décharges séparées par des intervalles de 29 msec.

| Intensité 2 | Choc unique 309–309 (9) | Choc double 292–292 (17) | Choc triple 192–288 (16) | Choc quadruple 289–283 (17) |

L'action devient moindre mais est encore sensible à la 4ème décharge (intervalle total de 87 msec.). Avec des séries continues de stimulations, l'action raccourcissante se montre en général plus grande qu'avec la double décharge. Chez le même sujet P, on obtient les valeurs suivantes des temps.

Intensité	Intervalle de 30 msec.		Intervalle de 80 msec.	
	Choc double	Chocs en série	Choc double	Chocs en série
2	265–261 (20)	282–280,5 (20)	293–303 (15)	281–287 (15)

Chez le sujet S voici les valeurs obtenues:

Intensité	Intervalle de 13 msec.		Intervalle de 40 msec.	
	Choc double	Chocs en série	Choc double	Chocs en série
1,25	286–280 (12)	246–242 (9)	299–310 (8)	264–246 (11)

L'action des décharges consécutives répétées peut donc être un peu plus grande que celle du double choc, le phénomène de sommation se traduit par l'accélération réactionnelle déclenchée dès le premier stimulus. Le fait du raccourcissement par les stimulations consécutives est en tout cas indéniable.

L'intervalle entre la première stimulation et la seconde, en cas de double décharge, peut être assez grand, mais à condition toutefois que les temps comportent encore une assez grande marge réductible. Au delà de 150–200 msec., on ne doit plus attendre d'action raccourcissante. Quand la marge réductible est épuisée, une réduction, du fait du double choc, ne peut naturellement plus être obtenue ou devient insignifiante (cas de l'intensité 3 chez le 1er sujet P, de l'intensité 1,44 chez le sujet H). Mais les données sont insuffisantes pour une analyse quantitative.

Le fait est que, de même que le seconde décharge peut augmenter rétroactivement l'efficience de la première, elle peut aussi raccourcir la latence de la sensation provoquée par cette première décharge. Comme la latence diminue lorsque croît l'intensité, il y a accord entre le fait de la facilitation et celui de la diminution de latence réactionnelle, qui témoigne d'une accélération du processus de l'éveil de la sensation. Tout se passe comme si, du fait de la décharge consécutive, l'efficience de la première décharge était augmentée de la même manière que par une élévation de son intensité objective.

V. Discussion et Essai d'Interprétation

L'efficacité d'une décharge brève de condensateur dont tous les faits d'enregistrement, sur l'animal, des potentiels d'action, permettent d'affirmer qu'elle engendre dans les fibres afférentes tactiles un influx unique, indique une possibilité d'éveil de la sensation n'impliquant pas un mécanisme itératif.

Lorsqu'à partir du seuil on augmente l'intensité de stimulation, à durée constante, ou la durée, à niveau constant d'intensité, on engendre une sensation de choc tactile plus intense, on met alors en jeu un nombre croissant de fibres et ce processus de recrutement progressif est à la base de l'accroissement d'intensité sensorielle; il y a d'ailleurs, lorsque le voltage augmente, une extension plus grande de la zone où se trouve localisée l'impression tactile, sur le territoire d'innervation de la branche sensitive excitée.

Quel que soit le mécanisme qui, dans l'excitation tactile physiologique, permet de différencier l'extension de l'excitation et son intensité, dans la stimulation électrique du nerf, les deux processus vont de pair. Mais, d'autre part la répétition de chocs successifs permet, à durée et niveau d'intensité constants de ces chocs, de provoquer une sensation plus intense (ou d'atteindre le seuil avec des stimuli isolément infraliminaires).

Dans la consécution de deux décharges, sous voltage égal, la seconde donne naissance à un choc tactile perçu plus intense. Cette sommation, indéniable, bien qu'elle n'ait pas été constatée par Schriever et Hegemann[2] est-elle de siège périphérique ou central? Gasser[3] a constaté, sur des nerfs vératrinisés ou préalablement soumis à une faradisation, que la répétition de stimulations électriques brèves pouvait entrainer des accroissements de potentiel témoignant d'un recrutement progressif de nouvelles fibres gagnées par l'excitation, et cela pendant une durée susceptible d'atteindre une demi seconde (gain au cours de l'application de 10 à 15 stimuli au rythme de 30 à la seconde). Mais, même si les nerfs tactiles, en place, soumis à des stimulations quasi-continues, pouvaient avoir des propriétés analogues à celles des nerfs préalablement faradisés, l'accroissement progressif d'intensité des chocs, perçus distincts (avec impression de type vibratoire), pourrait-il se confondre avec l'abaissement des seuils et le renforcement d'intensité constatés? Ce ne serait peut être pas impossible, s'il n'y avait cette facilitation rétroactive qui ne peut évidemment avoir son siège que dans les processus centraux.

Au niveau des centres, l'établissement progressif de l'état d'excitation préalablement au déclenchement d'une réponse répétitive des neurones synaptiques, observé par Barron et Matthews[4] dans la moelle, peut rendre compte d'un processus de sommation dans le temps, accéléré par action convergente de conducteurs afférents apportant simultanément les neuroquanta de leurs influx propres, ce qui permet de rendre compte d'une réduction de latence, alors même qu'il n'y aurait pas de réponse répétitive dans les fibres afférentes.[5]* La réduction, que nous avons observée, des temps de réaction, corrélative d'un recrutement progressif de fibres sensitives gagnées par l'excitation, pour les chocs isolés électriques (par décharge de condensateur de

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* Pour des stimulations prolongées, la marge réductible, avec l'intensité, des temps de réaction, est essentiellement due à la réduction du temps d'action liminaire. Mais il existe une marge réductible pour les stimulations brèves, interprétée par l'un de nous comme due à la réduction de l'intervalle d'un couple d'influx consécutifs quand la fréquence de la réponse s'élève avec l'intensité, le couple d'influx étant nécessaire pour le franchissement d'une synapse itérative.

durée chronaxique) quand croît l'intensité de ce choc (élévation de voltage) peut être ainsi élucidée. Mais la facilitation rétroactive n'est pas explicable par ce processus de l'établissement progressif de l'état d'excitation.

Nous avons été conduits à une hypothèse explicative qui a trouvé ultérieurement son appui expérimental dans les recherches de Lorente de No.[6] Sans avoir pris connaissance encore des études de ce dernier, une seule explication nous avait paru possible. La réponse de neurones synaptiques corticaux conditionnant l'éveil de la sensation tactile n'est pas encore engendrée aux environs du seuil quand ces neurones sont atteints par des influx suivant une voie d'accès directe, l'apport de neuroquanta étant insuffisant pour provoquer un niveau de l'état d'excitation correspondant au déclenchement de la réponse propre de ces neurones de type lent. Il faut que des influx, suivant une voie polysynaptique plus longue, viennent à leur tour converger sur les neurones en question, et compléter l'apport liminaire des neuroquanta nécessaires pour que la réponse se produise et que la sensation naisse—que le *mécanisme de sommation réside en des modifications électriques cumulatives* ou comporte un processus neurohumoral de libération progressive d'acétylcholine.

L'apport par voie directe—san doute nécessaire—est préparateur et facilitant, bien qu'inefficace. Si l'on est au dessous du seuil, même l'apport ultérieur, retardé, des voies polysynaptiques, est inefficace, la convergence ne fournissant qu'un nombre total encore insuffisant de neuroquanta. Mais si, entre l'arrivée des influx directs, et celle des influx retardés, survient une seconde volée d'influx directs, les influx retardés deviendront efficaces, *complétant cette fois le niveau d'action liminaire par l'apport de leurs neuroquanta*, et la sensation, qui n'était pas apparue avec le choc unique, sera déclenchée dans les mêmes conditions que si un accroissement d'intensité du stimulus avait permis, par action convergente d'un plus grand nombre de fibres, de rendre efficace l'apport retardé de neuroquanta par les voies polysynaptiques.

L'action, d'apparence rétroactive, s'explique en ce qu'elle s'insère pendant la phase de latence centrale au cours de laquelle s'élabore un état d'excitation par apport successif de neuroquanta suivant des voies polysynaptiques plus ou moins complexes, et réalisant l'itération répétitive qui n'existe pas dans les voies afférentes précentrales, précorticales même peut-on dire.

Les deux chocs successifs seront rendus efficaces, mais avec leur intervalle normal, car, pour la deuxième réponse, facilitée au niveau de neurones qui ne sont pas nécessairement les mêmes que ceux qui ont répondu au premier choc—par les apports préalables, elle ne se produira aussi que lorsque parviendront les influx retardés des voies polysynaptiques correspondant à la seconde volée d'influx afférents. On peut voir, sur la Fig. 2 ci-jointe, un schéma—évidemment très simplifié—de l'évolution de l'état d'excitation, en distinguant l'apport direct et l'apport retardé qui permet de rendre compte de la double réponse quand, à un niveau infraliminaire, la répétition du stimulus assure son efficacité. On y comprend également l'action accélératrice qu'exerce

le second choc, diminuant la latence réactionnelle à une même intensité de stimulation. En effet l'action facilitante exercée par l'apport suivant la voie directe qu'assure le second choc entraine, pour un même niveau d'intensité, un franchissement plus rapide du seuil de la réponse répétitive des neurones[*] lorsque surviennent les influx retardés des voies polysynaptiques.

FIG 2 Schéma de l'évolution de l'état d'excitation d'un esthésioneurone sous l'influence d'un choc électrique unique ou double porté sur une branche cutanée sensitive Un influx direct infraliminaire est suivi d'une volée polysynaptique Les traits placés en dessous indiquent les chocs électriques, dont l'intensité est marqué par la hauteur du trait. Les traits situés au dessus indiquent les influx déclenchés par l'esthésioneurone En I, choc unique infraliminaire En II choc juste liminaire, en III, choc supraliminaire En IV double stimulation par chocs de même intensité qu'en I, l'insertion de l'influx direct du deuxième choc permet à la volée polysynaptique du premier choc d'atteindre le seuil d'excitation efficace, la deuxième volée polysynaptique survenant à son tour dépasse légèrement le seuil En V, double stimulation par chocs de même intensité qu'en III. L'insertion de l'influx direct du 2° choc avant l'arrivée de la première volée polysynaptique accélère le franchissement du seuil d'excitation et accroit l'efficacité de la première réponse, la seconde atteignant un niveau un peu plus élevé que la première
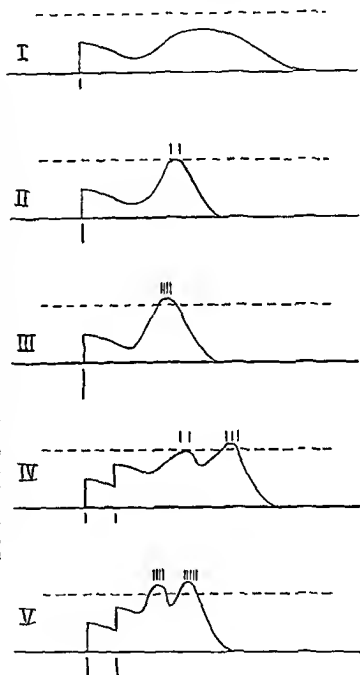
Le schéma se contente d'indiquer un apport retardé un peu massé, mais il est possible que les apports retardés se succèdent sur une période relativement longue, et qu'ainsi la réductibilité de latence puisse varier de façon continue dans une marge assez grande, marge réductible qui, déterminée par les mesures

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[*] Avec un choc électrique unique donnant sur le nerf optique—homologue d'une voie médullaire sensitive—un seul potentiel d'action, on recueille, dans l'écorce striée, une réponse répétitive d'une certaine durée (réponse de 20 msec , accrue par la strychnine d'après les résultats de Bartley, O'Leary et Bishop).[7]

de temps de réaction se montre de l'ordre du dixième de seconde. D'autre part le fait que la sensation vibratoire peut être encore perçue à des fréquences assez élévées donne à penser que, parmi les esthésioneurones en connexion avec les fibres afférentes, un certain nombre répondent à chaque stimulus de choc, les autres étant en phase réfractaire, en sorte que le processus du double choc perçu puisse évoluer dans des neurones différents pour le premier et pour le second, l'arrivée des influx, suivant les voies polysynaptiques, qui aboutissent à plusieurs neurones, étant toujours nécessaire pour déclencher la réponse, aussi bien des neurones excités par le premier que de ceux excités par le second choc.
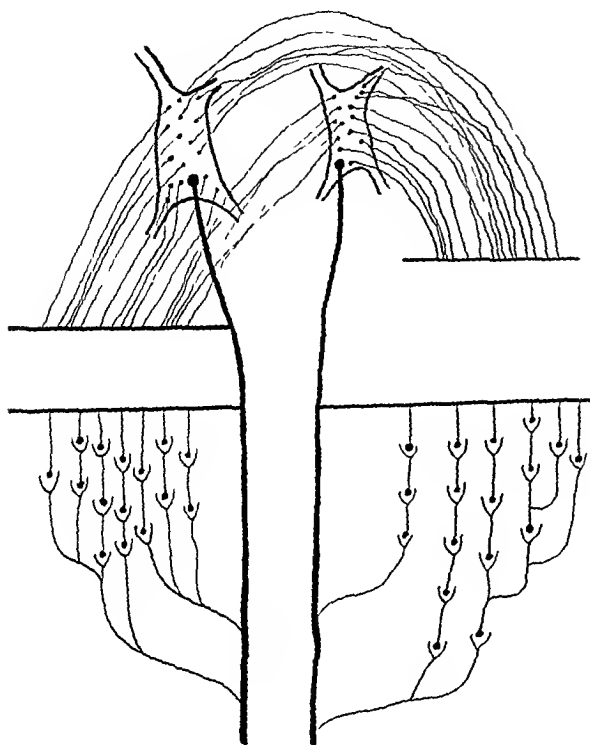
FIG. 3. Schéma relatif aux voies afférentes aboutissant à deux esthésioneurones corticaux, avec voie directe spécifiquement liée à un neurone défini, et voies dérivées polysynaptiques se rendant à l'un et l'autre neurone après un nombre variable de passages cellulaires entraînant des retards dans l'apport des influx (un grand nombre de synapses non indiquées correspondant à la coupure du schéma).

L'interprétation proposée s'accorde avec les faits étudiés par Lorente de Nó dans la stimulation des voies encéphaliques de la motricité oculaire du lapin, mettant en évidence l'arrivée retardée d'un bombardement d'influx par des voies polysynaptiques de neurones "internunciaux," de paradosioneurones Les chaines multiples de ces cellules interposées constituent, dans les centres cérébraux—et particulièrement dans l'écore—les véritables unités élémentaires de transmission, selon Lorente de Nó. Si les durées du délai dans l'excitation des noyaux oculomoteurs du lapin sont relativement brèves, ces durées, en ce qui concerne les polysynapses de l'homme peuvent être beaucoup plus longues.*

Au niveau de l'épanouissement principal des fibres afférentes sensitives en

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* Des durées longues de facilitation s'observent dans les nerfs amyéliniques de Crustacés où l'on peut noter, comme dans les centres, des variations lentes de potentiel caractéristiques de l'état d'excitation, étudiées par A. Arvanitaki,[8] qui a obtenu par exemple (effet pseudo-réflexe) une décharge répétitive afférente provoquée par sommation de 2 influx afférents isolément inefficaces, avec un intervalle de 0,24 sec.

d'innombrables ramifications, dans les couches moyennes du cortex, celles des grains, il existe des myriades de neurones, à interconnexions limitées à ce niveau (cellules en étoile), qui doivent intervenir dans ces relations poly-synaptiques, tandis que d'autres cellules, moins nombreuses, du type pyra-midal, assurent les connexions avec les régions supérieures du cortex et les transmissions conditionnant les processus perceptifs et l'intégration des ex-citations sensorielles dans le comportement psychologique.

Sans prétendre attribuer à des couches définies du cortex des fonctions absolument exclusives, attribution exclusive contre laquelle s'élève Lorente de Nó,[9] il paraît légitime, cependant, en l'état actuel de nos connaissances, d'attribuer, aux couches moyennes rôle important dans les processus de récep-tion corticale, et les phénomènes sensoriels initiaux. Mais, après passage par les chaines polysynaptiques dont la réalité histologique est indéniable, l'éveil de la sensation, perçue avec retard, se fait-il dans les couches supérieures du cortex de projection, ou dans des régions avoisinantes c'est ce qu'il n'est pas possible d'affirmer à l'heure actuelle.

CONCLUSION

Le processus de facilitation rétroactive que nous avons mis en évidence avec un mode de stimulation relativement simple constitue en quelque sorte la contre partie de l'inhibition rétroactive qui, dans le domaine plus complexe des stimulations visuelles caractérise le métacontraste, découvert par Stigler, retrouvé par Fry, et systématiquement étudié par l'un de nous.[10,11,12]

Dans le métacontraste, il se produit, au niveau des centres ganglionnaires de la rétine, un raccourcissement de la réponse provoquée par une stimulation lumineuse locale quand survient ensuite—dans une certaine marge d'inter-valles—une autre stimulation adjacente. Mais la sensation qui naît de la première excitation, raccourcie, peut être entièrement supprimée dans cer-taines conditions, et faire pleinement défaut. On peut penser que ce processus cortical d'inhibition est, lui aussi, rendu possible, grâce à cette phase de latence qui s'écoule entre l'arrivée des influx afférents directs et celle des influx différés suivant des voies longues polysynaptiques et dont le bombardement est nécessaire pour établir, dans les esthésioneurones, un niveau de l'état d'excitation (qui se traduit, d'après les données de Matthews, par une polari-sation durable) suffisant pour déclencher la réponse conditionnant l'éveil de la sensation perçue.

D'autre part la marge réductible du temps de réaction qui, dans le cas de réponses afférentes répétitives, peut être ramenée à une réduction d'intervalle entre deux influx nécessaires pour le franchissement d'une synapse à caractère itératif,[13] devient explicable, même dans les cas où la première interprétation n'est plus possible,* en particulier quand, sans itération, se montre efficace pour éveiller une sensation le déclenchement d'un seul influx afférent, comme

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* Des expériences sur les temps de réaction à des accroissements de brillance plus ou moins intenses, à partir de niveaux variables, se sont montrées incompatibles avec l'in-terprétation fondée sur la réduction de l'intervalle dans un couple d'influx.[14]

dans nos expériences de stimulation de fibres tactiles par un choc électrique bref.

C'est l'intervention des voies polysynaptiques de transmission cérébrale qui introduit une itération nécessaire, de durée réductible, lorsqu'un plus grand nombre de voies afférentes, se distribuant en un groupe d'esthésioneurones, sont simultanément mises en action par une stimulation plus intense. Ce bombardement, par apport des voies polysynaptiques, devient plus vite efficace, réalisant plus tôt le niveau de l'état d'excitation nécessaire pour que soit déclenchée la réponse conditionnant l'éveil de la sensation perçue, et que se produise le geste réactionnel que cette perception commande.

Le paradoxe de la rétroactivité fait place à la notion d'influences s'exerçant au cours de l'élaboration retardée de l'état d'excitation des esthésioneurones, en raison d'une participation nécessaire (sauf peut être dans des états d'hyperexcitabilité tels que ceux qu'engendre la strychninisation) des influx différés suivant les voies polysynaptiques corticales.

RÉSUMÉ

Dans la stimulation par décharges brèves de condensateurs d'une branche sensitive cutanée (rameau digital du médian), chez l'homme, il a été constaté que, pour un niveau d'excitation infraliminaire, l'addition d'une seconde décharge consécutive, dans une assez grande marge des intervalles (dépassant le dixième de seconde) assurait la perception du choc tactile provoqué, non seulement par la deuxième, mais aussi par la première décharge, grâce à un processus de facilitation rétroactive. Cette facilitation s'est révélée encore par un raccourcissement des temps de réaction au choc tactile sous l'influence de l'intervention consécutive d'une seconde décharge, dans une marge analogue d'intervalles. L'interprétation de ce phénomène, d'apparence paradoxale, repose sur l'intervention d'un retard d'élaboration de la réponse des esthésioneurones conditionnant l'éveil de la sensation perçue par intervention nécessaire d'influx suivant des voies polysynaptiques—dont Lorente de Nó a établi l'existence par ses recherches histologiques et physiologiques—influx dont le bombardement débute un certain temps après l'arrivée d'influx directs inefficaces. A un niveau infraliminaire, le second choc, survenant entre l'influx direct et les volées retardées, peut rendre efficace le premier stimulus grâce à l'intervention de son influx direct, quand surviennent les influx qui déclenchés par le premier ont suivi les voies polysynaptiques; et, à un niveau supraliminaire il accélère l'établissement d'un niveau efficace et diminue ainsi la latence.

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ABLATIONS OF FRONTAL CORTEX IN CATS WITH SPECIAL REFERENCE TO ENHANCEMENT OF THE SCRATCH REFLEX

F. KEITH BRADFORD

From the Division of Neurology and Neurosurgery, University of Chicago

ALTHOUGH numerous investigators have studied cats following extirpation of various parts of the cerebral cortex, especially the area about the cruciate sulcus, their accounts make no reference to certain reflexes and modifications of behavior herein described. Emphasis will, therefore, be placed upon these phenomena, and findings corroborating previous investigators will only be mentioned as they relate to these data.

METHODS

Pentobarbital sodium, 25 to 35 mg. per kg. intraperitoneally, was the only anesthetic used. All operations were conducted under aseptic conditions. A spatula was used for the subpial dissection. The frontal sinuses were opened widely on one or both sides except in the operation on Cat 118. The frontonasal ducts were occluded with muscle before closing. Only one operation was performed on each cat. It was found most convenient to use a horsehoe shaped incision with the base toward the forehead. The cats were sacrificed by administering pentobarbital sodium, 50 mg. per kg. intraperitoneally, and opening the chest in order to perfuse the brain *in situ* with physiological saline followed by 10 per cent formalin. After further fixation in formalin, dehydration, and embedding in nitrocellulose, serial sections were cut 40 μ thick. Every 30th section was stained by Weil's[13] technic for myelin.

RESULTS

Bilateral Preparations

In 4 cats (Nos. 119, 121, 124, and 125) the frontal cortex on both sides was extirpated, including in each instance the sigmoid gyri, most of the gyrus proreus, and part of the area surrounding the sigmoid gyri. Since the post-operative behavior of all 4 cats was similar, they will be described first as a group. In none was it possible to elicit the 4 placing reactions of Bard[1] during the survival period. The hopping reactions were markedly impaired. For 1 or 2 days it was necessary to feed these cats by spoon, but soon they ate spontaneously. After a week of untidiness they groomed and soon could not be distinguished from normal cats by their coats. None of the animals showed "sham rage," but at times during the first postoperative month all except Cat 121 showed momentary spitting and fighting, poorly directed and lasting but a few seconds. All 4 cats were in almost constant motion, their tails in particular being abnormally active. The cutaneous maximus muscle frequently twitched, especially when the vertebral column was tapped.

Cats 121 and 124 showed postoperatively for 2 months almost constant purring which then became less frequent. Mistreatment tended to increase

the purring which, in the latter months of survival, occurred only after handling, usually of an unpleasant sort. Strangely, purring was never observed in Cats 119 and 125. These 4 cats tended to watch an object rubbed across the wire of the cage as long as it was kept in motion. When they were allowed to run loose, they almost constantly followed a moving person, but if he stopped interest appeared to be lost. The response to petting was diminished. They were easily handled, but became restive upon being held. *Gait* was severely disturbed for the first few days. It was usual to see one of these cats fall after shaking. Another temporary disturbance was the placing of a forefoot on the dorsal rather than on the plantar surface. From the time of operation on, all 4 cats showed increased extensor tonus in all extremities. The tonic neck reflexes of Magnus[8] were found transiently in but one cat (No. 119). During the entire survival period these cats kept their claws out more than normally, frequently getting them hooked in the wire cage. On being held in the supine position, the body and neck were flexed forward and then the forelegs with claws extended clutched at the abducted hind legs, pulling the shoulders through. The hind legs were next brought into a standing position, and the animal walked off.

Scratch reflex. Cats 121, 124, and 125 showed a remarkable accentuation of the scratch reflex. Cat 119 was not examined for the scratch reflex since death occurred before the increase in the reflex was observed in Cat 121. In cats the scratch reflex is elicited in the same manner as the auriculo-genital reflex.[3] Tweaking the cartilaginous portion of the external ear between the forefinger and thumb from behind, or rapidly rotating the index finger or a cotton swab placed in the external acoustic meatus was the most satisfactory method of eliciting it. Stimulation of parts of the head and neck other than the external ear, or stimulation of the chest and flank never produced the scratch reflex in either normal or operated cats. Faradic stimulation produced a response only when applied to the skin of the external acoustic meatus.

Cat 121 showed the most remarkable increase in the scratch reflex which became apparent 2 weeks after operation and persisted until the animal was sacrificed 3 months later (Fig. 1). Spontaneous scratching resulted in ulceration of the skin over the occiput and back of the neck. In Cat 125 the scratch reflex became hyperactive on the tenth postoperative day, and in Cat 124 only after 6 postoperative weeks. The hyperactivity of the scratch reflex was out of all proportion to that in normal cats. Irrespective of the cat's position, stimulation of one ear would cause flexion of the trunk and neck to that side with effective and continued scratching by the homolateral hind leg. With the cat held in the erect position by the head and one ear stimulated, the homolateral hind leg was lifted from the floor, undergoing rhythmic scratching movements which in this case were ineffective. If both external ears were stimulated simultaneously, there were clonic contractions of both hind legs joined later by the forelegs. Then the head drew back simulating a clonic convulsion which lasted only during the period of stimu-

lation. This caused marked restlessness and miaowing. In normal cats such maneuvers as the last caused only a display of anger, and stimulation of one ear elicited the scratch reflex only when the position in which a cat was lying was convenient for scratching. Even so, the scratching was frequently abortive, contrasting strikingly with the sustained compulsory scratching of the operated cats which would struggle awkwardly from side to side, scratching alternately with the hind legs as the ears were alternately stimulated.

Auriculo-genital reflex. When the external ear was stimulated in one of
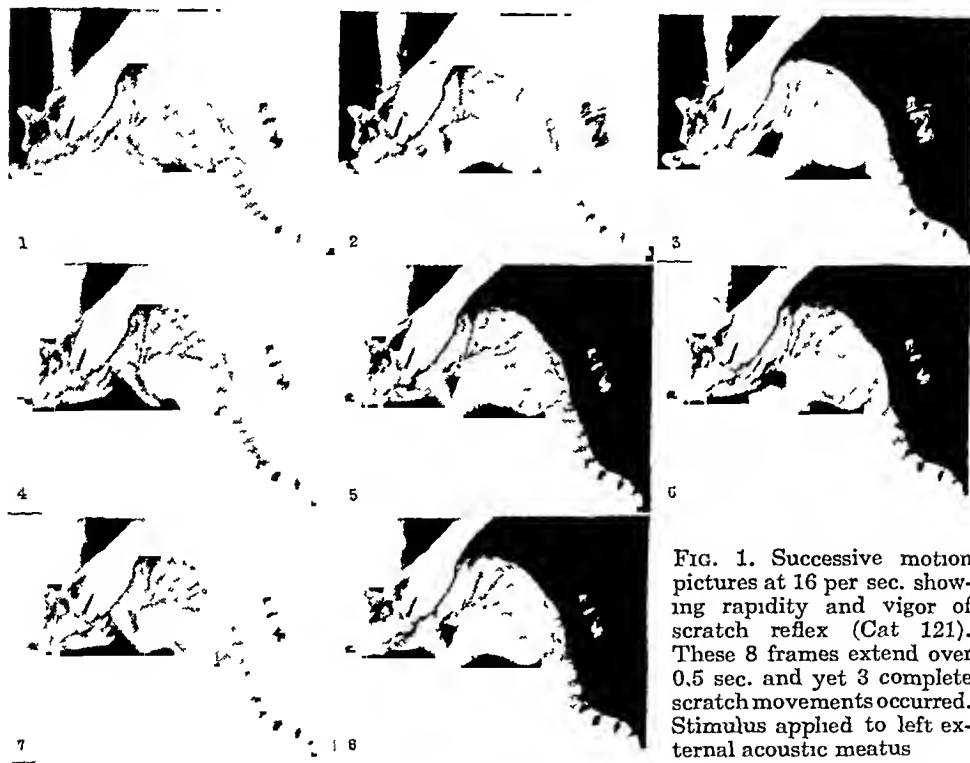
FIG. 1. Successive motion pictures at 16 per sec. showing rapidity and vigor of scratch reflex (Cat 121). These 8 frames extend over 0.5 sec. and yet 3 complete scratch movements occurred. Stimulus applied to left external acoustic meatus

the ways just described to elicit the scratch reflex, there also resulted contraction of the subcutaneous muscle about the penis or vagina.[3] This response, present in normal cats, could be elicited far more easily in Cats 121, 124, and 125 than in normal animals, although there was great variability in the ease of elicitation in the normals. Brushing the hand across the ear in a manner to fold it forward and downward frequently elicited the reflex in the operated animals although this was an inadequate stimulus in normal cats.

"Going-under-or-over-fence" reaction. In Cat 121, and to a lesser degree in Cats 124, and 125, a reversal of the usual reaction to gentle stroking of the back was observed. Cat 119 was not studied for this response because

it was not observed until after this cat's demise. A normal cat almost invariably arches its back when gently stroked. But these cats did just the opposite, making the back concave beneath the stroking fingers. This response was obtained on the 12th postoperative day, continuing throughout the survival period in Cat 121, although during the last month it was obtained only when the cat was in motion. As the cat moved beneath the gently stroking finger the back was lowered markedly, and as the sacrum came under the finger the pelvis was lowered almost to the floor and the hind legs clambered awkwardly behind the animal in a manner identical to that of a cat crawling through a hole under a fence (Fig. 2). If the undersur-
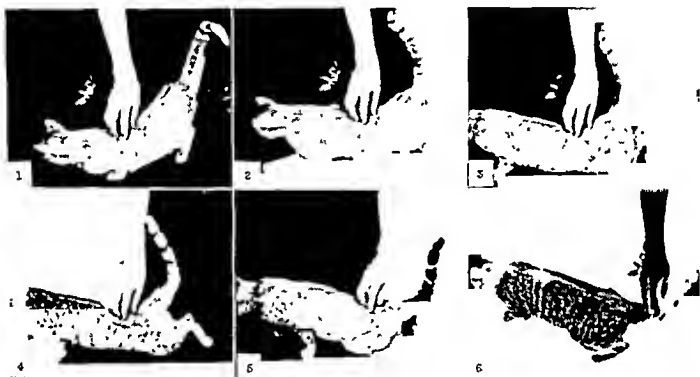
FIG. 2. Six frames from a motion picture have been selected to demonstrate the "under fence" reaction in Cat 121. They are reproduced in order, representing a 2 second period. The fingers can be seen touching the back lightly, and yet the response is as if the cat were trying to pass under a fence.

face of the abdomen was gently stimulated with the finger the back was arched greatly, and if the cat was in progress, the hind legs were raised awkwardly as if to clamber over a board when this point was reached.

"*Climbing discharge.*" With the exception of No. 121, these 4 cats exhibited an unusual type of motor discharge during the first few postoperative days lasting up to 2 weeks. When these cats were held upright and fed with a spoon, they settled back in a characteristic position on their haunches and for several mouthfuls would swallow well enough. But often upon continuing this, they became restless, the pupils dilated, and jumping movements occurred. If this discharge was encouraged by jumping the animal up and down holding it by the chin and scruff of the neck, the springing gained in extent and was accompanied by increasingly violent climbing movements of the forelegs. If, at the height of the discharge, the cat was

held with feet toward the side of a wire cage, it rapidly but awkwardly climbed up. To keep it from falling backward it was necessary to retain a grip on the scruff of the neck, for the climbing movements were not sufficiently accurate to hold constantly to the wire cage. Cat 124 voided once at the termination of such a discharge. At no other time during the survival periods did any of these 4 cats attempt to climb the wire cage, nor, after 2 weeks postoperative, could such a reaction be produced. The "climbing discharge" appeared identical to that observed in several unanesthetized cats when bipolar electrodes implanted in the hypothalamus were stimulated with faradic current.

Groping and grasping. For the first postoperative week Cat 125 showed definite groping and grasping in the forelegs when held in an upright position on its haunches. One forepaw would make rhythmic groping movements. If it came in contact with something, it would be brought strongly down toward the cat's side. Then the other forepaw would similarly grope and only be brought toward the body if it met an object in its groping. Although the groping and grasping were constant from the 2nd to the 7th postoperative day, many subsequent attempts to elicit this reaction failed.

Licking and biting response. Cat 124 showed a peculiar reflex elicited by scratching over the sacrum just anterior to the base of the tail. Whenever stimulation was made in this manner, the cat would begin to lick the air, the floor, or even lick and bite his own forelegs. Bard and Rioch[2] have described such a reflex in decorticate animals, which was useful in getting them to eat. But in their decorticate cats the reflex was obtained from stimulation anywhere on the cat's body. The small area over the sacrum was the only area from which the reflex could be elicited in this cat and, after it was discovered about 3 weeks postoperative, the licking and lapping response never failed to appear upon stimulation here. Olmsted and Logan[10] mention a reflex occurring in a female cat with destruction of rostral cortex on both sides which was elicited from the same area as was the "licking response." This consisted of elevation of the tail and flexion of the hind legs into a squatting position. This was interpreted as a sexual reflex.

With the exception of Cat 119, these 4 cats lived until sacrificed. Cat 119 ate well and kept its coat neat and clean until the 33rd postoperative day when it was observed circling wildly around the large cage it occupied with several other cats. The pupils were maximally dilated, giving a wild, frightened appearance. When Cat 119 was separated from the other cats, the violent overactivity continued. Even *in extremis* there were occasional violent outbursts of activity. The cat expired the following day. Postmortem examination disclosed only the expected ablation without evidence of any operative complication. Cats 121, 124, and 125 were allowed to survive 93, 162, and 161 days respectively.

Extent of ablations. In Cat 119 destruction on both sides included rostrally the anterior and posterior sigmoid gyri and the third of the gyrus proreus proximal to the anterior sigmoid gyrus (Fig. 3). Posteriorly and

laterally there was destruction of the coronal gyrus, the anterior suprasylvian gyrus, and the anterior part of the gyrus lateralis on the right and to a slightly lesser extent on the left. Descending degeneration could not be followed with a myelin sheath stain because of the relatively short survival period (34 days).

The ablation in Cat 121 was like that of Cat 119 except that on the left the gyrus proreus was removed but for a small ventral fragment, and there was slight damage to the rostral tips of the caudate nuclei. Coronal sections stained for myelin showed on each side a compact degenerated bundle which could be followed from beside the head of the caudate nucleus into the medial half of the posterior limb of the internal capsule. The degenerated
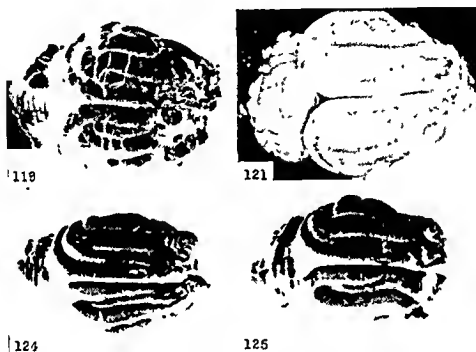
FIG. 3. Dorsal view of the brains of Cats 119, 121, 124, and 125 showing in each the bilateral ablation of rostral cortex.

fibers were rearranged in the cerebral peduncle to form a crescent next to the substantia nigra. In the pons the degenerated bundles were situated laterally and toward the tegmentum. The pyramids of the bulb were completely degenerated and severely atrophied.

In Cat 124 the ablation included bilaterally most of the gyrus proreus while in Cat 125 only one third of each gyrus proreus was included. The myelin degeneration in both resembled closely that occurring in Cat 121.

Unilateral preparations

Three cats with operations limited to the right side (117, 118, and 120) are included in this report chiefly to corroborate the observations of previous investigators. Cats 117 and 118 behaved so similarly that they can be described together. There was permanent increase in the extensor tonus of both left extremities as demonstrated by supporting the animal by head

and tail or by placing it in the supine position with the neck extended. From the first postoperative day throughout survival the placing reactions were absent in the contralateral (left) extremities. There was attitudinal abnormality of the affected extremities and failure to use them properly in leaping from tables, etc. Both cats showed a response to petting on the right side only. The scratch reflex could not be eliectied from the left ear (side opposite the ablation) for the first few weeks postoperative although easily elicited from the right ear. However, after 2 months the reflex was as great or sometimes greater when elicited from the contralateral (left) ear. In addition, Cat 118 showed the "climbing discharge" as described for the bilaterally operated cats for several days during the second postoperative week. The "fear reaction" at the hissing sound of escaping air was also present. In retreating from this sound, Cat 118 once wedged its head firmly beneath a refrigerator.

Cat 120 had a very small cerebral ablation limited to the sigmoid gyri on the right side. The contralateral foreleg showed complete absence of the placing reactions, but the hind leg was not entirely deficient although it contrasted markedly with the homolateral hind leg. The left foreleg showed more constant attitudinal abnormality than the left hind leg. Personality and response to petting were unchanged. The scratch reflex elicited from the homolateral (right) ear was active, from the contralateral (left) ear at first absent, until they became equal about 2 months postoperative. Cats 117, 118, and 120 were allowed to survive 245, 174, and 633 days respectively.

Extent of ablations. In Cat 117 destruction limited to the right side included the anterior and posterior sigmoid gyri, the adjacent half of the gyrus proreus, the coronal gyrus, the anterior suprasylvian gyrus, and the anterior part of the lateral gyrus. Coronal sections stained for myelin showed degeneration limited to the right side similar to that described for the bilaterally operated animals. In Cat 118 the ablation was similar to that in Cat 117, but (probably as a result of pressure on the anterior part of the hemisphere to bring about hemostasis) cystic degeneration of the hemisphere occurred, leaving intact only a small portion of the temporal and occipital cortex. Sections stained for myelin revealed complete degeneration of the internal capsule, cerebral peduncle and pyramid on the affected side. The small ablation in Cat 120 was limited to the anterior and posterior sigmoid gyri on the right but did not include all the cortex buried in the cruciate sulcus. The degeneration was less marked than in the other cats. The right pyramid was incompletely degenerated, but atrophied to about half the size of the left.

DISCUSSION

The scratch reflex in the cat can be readily elicited only from the region of the external ear, the same area from which the afferent end of the reflex arc for the auriculo-genital reflex takes origin. These 2 reflexes differ widely in their activity in normal cats. Since the auriculo-genital reflex is frequently

very active in normal cats, it is difficult to judge finally in regard to its exaggeration. On the other hand, the scratch reflex, although variable, is partially inhibited in normal cats, making accentuation clear when it does occur.

Cats 117, 118, and 120 showed a temporary absence of the scratch reflex when attempt was made to elicit it from the ear opposite the ablation, although it could be elicited from the homolateral ear. This may have been merely a coincidence since the response to stimulating the ears later became equal. It is mentioned because the bilaterally operated cats (121, 124, and 125) underwent a period of 10 days or longer when the scratch reflex could not be obtained after operation. It is rather to be suspected that one frontal pole will maintain an inhibitory activity on underlying centers having to do with activity of the extremities on both sides. Fulton[4] and his coworkers have shown in monkeys that area 4 or 6 preserved on one side alone influences the tonus and reflex activity of the homolateral as well as the contralateral extremities.

Bard and Rioch[2] report that rubbing the side of the head of a decorticate cat caused, at times, turning of the head to that side followed by attempts to scratch with the ipsilateral hind foot. If the ear had been stimulated rather than merely the side of the head, exaggeration of the scratch reflex would probably have been observed in their decorticate cats similar to that observed here in cats with ablated frontal poles. Olmsted and Logan,[10] King,[6] Langworthy,[7] and Magoun and Ranson[9] have reported the motor findings in cats with similar bilateral ablations of rostral cortex, but no reference is made to the scratch reflex.

The "climbing discharge" evidently represents a discharge of lower centers, probably hypothalamic. The reaction is certainly a purposive one, although disorderly. It was present only in the immediate postoperative period. This reaction corresponds closely to that reported by Rioch and Brenner[11] from stimulation of the ventricular floor at and in front of the anterior commissure in cats 10 days or longer following decortication. In their cats 5 to 10 seconds of faradic stimulation was followed by a sudden burst of activity in the form of violent springing and running movements continuing for one minute after stimulation had ceased. Two explanations of the "climbing discharge" seem possible. First, the fibers degenerating as a result of the ablation may have influenced diencephalic centers in a way to make them more irritable for a period not greater than 2 weeks. Second, other inhibitory mechanisms, cortical or subcortical, may have replaced the ablated area in its inhibitory activity at the end of 2 weeks. The transient groping and grasping observed in Cat 125 must likewise have been due to transient overactivity of lower centers, probably hypothalamic.

The "going-under-or-over-fence" reaction represents the uncontrolled activity of a useful mechanism. The movements resemble closely those of a normal cat going through a hole under a fence or clambering over a board. But the gentle stroking of the back with one finger in an anteroposterior

direction necessary to elicit the "under-fence" reaction in the bilaterally operated cats caused arching of the back in normal cats. Since these reactions diminished in the ease with which they were elicited during the last weeks of survival, it seems probable that some other inhibitory mechanism gradually replaced the ablated rostral cortex. Bard and Rioch[2] described behavior in one cat after bilateral removal of neocortex which somewhat resembles the "under-fence" reaction, but may be related to sexual activity. When its back was rubbed or scratched this cat relaxed, abducted the hind legs, depressed its tail, and dragged itself forward with the forelegs, growling loudly.

A stereotyped licking response was noted by Schaltenbrand and Cobb[12] and substantiated by Bard and Rioch[2] in decorticate cats. The reflexogenous zone included the neck, shoulders, back, and genital regions. It is interesting that this same response was present in Cat 124 but not in the other 3 bilateral frontal pole ablations, and in Cat 124 could be elicited only over the dorsum of the sacrum near the base of the tail. However, when elicited here, the reflex was fully developed.

Purring in response to petting or fondling is reported in decorticate cats by Schaltenbrand and Cobb[12] and by Rioch and Brenner.[11] In a series of 4 decorticate cats Bard and Rioch[2] report that purring was observed in only one cat, and then on only one occasion. Gibbs and Gibbs[5] report purring as a result of electrical stimulation in the infundibular region in 3 of 400 cats stimulated. Purring, however, in Cats 121 and 124 was almost constant during handling for the first 2 months postoperative after which it was less frequently observed. Rough handling or the deliberate pinching or roughing of these cats increased rather than decreased the intensity of the purring. Cats 119 and 125 with similar ablations, but with less destruction of the gyrus proreus on each side, were never observed to purr. It is possible that the ablation of both frontal poles released other undisturbed cortical areas from inhibition, or that diencephalic centers were similarly released. In the latter case the absence of purring in Bard's decorticate cats is not explained.

SUMMARY

1. Ablation of frontal cortex around the cruciate sulcus in cats produced in the bilateral experiments the behavior described by previous investigators including the absence of the placing reactions. In the unilateral experiments the behavior was unchanged, but the placing reactions were destroyed in the contralateral extremities.

2. The "climbing discharge," groping and grasping, the licking and biting response, exaggeration of purring, and the "under-or-over-fence" reaction were correlated with the observations of previous investigators.

3. Marked enhancement of the scratch reflex which occurred 2 to 6 weeks after bilateral frontal ablations persisted throughout survival periods up to 162 days.

4. Unilateral ablation of frontal cortex effected no enhancement of the scratch reflex, but only transient abolition in the contralateral extremities.

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TEMPERATURE REGULATION IN CATS WITH THALAMIC LESIONS*

GEORGE CLARK, H. W. MAGOUN, AND S. W. RANSON

From the Institute of Neurology, Northwestern University Medical School, Chicago, Illinois

IT IS now generally agreed that the hypothalamus plays an important part in temperature regulation (Ranson and Magoun, 1939). The remainder of the diencephalon does not appear to participate in this function, but it has seemed desirable to secure additional information on this point.

METHODS

With the aid of the Horsley-Clarke instrument, moderate sized lesions were placed bilaterally in various parts of the thalamus in 9 cats. In 6 other cats large lesions were made and in 3 of these in order to secure the maximum destruction without killing the animals, the operation was performed in two stages, first on one side and then the other side of the thalamus, with a period of 8 to 16 days intervening. A needle-like bipolar electrode with the bare tips of the constituent wires separated by 2 mm. along the long axis of the needle was used and through it a direct current of 3 mA. was passed for 1 minute to produce a lesion. In the 3 cats in which the greatest injury was inflicted 9 punctures were made on each side and 3 lesions were placed along each of the 3 lateral punctures and 2 along each of the other 6. The 21 lesions all fused together to form one very large lesion which after the second operation was united with a similar one on the opposite side.

Daily records were made of the rectal and environmental temperature and at varying times after the operation tests were made to determine the ability of the animals to withstand heat and cold. These hot and cold box tests were similar to those described by Teague and Ranson (1936) except that in the hot box a fan was provided to keep the air in circulation. The temperature of the hot box was 104°F. and that of the cold box varied considerably but averaged around 40°F. Before they were sacrificed each of the 6 cats with large lesions were decorticated under ether and as they recovered from the anesthetic they were watched for decorticate panting. They were then killed by bleeding and the brains perfused with 10 per cent formalin. Serial sections were cut through the diencephalon and alternate sections were stained by Weil's method for myelin sheaths and cresyl violet for cells.

RESULTS

The 9 cats with moderate sized lesions may be considered together as group A and the 6 with large lesions as group B. In both groups the rectal temperatures were above the normal average on the first postoperative morning. In group A the temperatures were around or slightly above the upper limits of normal. In group B the 3 cats (13, 14, 15) with the largest lesions had temperatures ranging from 104.4 to 105.1°F. on the first morning following each of the 2 operations. In no case was a subnormal temperature encountered. By the third day the cats began to eat voluntarily. The 3 cats in which the damage was at first unilateral circled to the side opposite the lesions for at least 3 days following the first operation. In none of the cats was there any indication of catalepsy or somnolence.

The results of the hot and cold box tests are given in Table 1. At the head of the table are the average results obtained from 94 tests performed on normal cats. While the average panting level, or rectal temperature at which panting began was 103.2°F., variations between 105.4 and 101.4°F. were encountered in normal cats. The table shows that all the cats of both groups panted in response to heat and that although the panting level was in most instances higher than the average for normal cats it was in each case

Table 1

Cat no	Hot Box Tests				Cold Box Tests				Decortications	
	Days PO	Panting level	Rise above initial temp.	Final resp rate	Days PO	Rectal temp at start of test (F)	Rectal temp at end of test (F.)	Change	Highest resp rate	Panting
Normal averages		103 2	1 6	214		101 2	101 0	−0 2		
Group A										
1	6	104 1	1 4	160	9	101 9	100 4	−1 5		
2	6	103 8	1 8	210	9	103 1	101 9	−1 2		
3	15	103 6	3 4	150	16	100 6	99 8	−0 8		
4	31	103 9	2 0	130	30	101 9	100 2	−1 7		
5	19	103 6	2 2	300	18	101 2	101 4	+0 2		
6	53	104 2	2 7	180	53	102 1	100 2	−1 9		
7	44	105 1	3 9	130	60	101 2	100 4	−0 8		
8	49	103 8	2 2	190	49	101 4	101 1	−0 3		
9	59	103 0	1 8	180	58	101 6	100 0	−1 6		
Group B										
10	13	103 1	2 0	160	14	102 3	100 4	−1 9	44	no
11	18	104 2	1 6	210	21	102 2	100 1	−2 1	166	yes
12	13	103 2	1 0	180	16	101 9	101 0	−0 9	156	yes
13	27	103 1	1 1	160					120	yes
14	48	103 5	1 1	150	17	102 3	102 0	−0 3	120	yes*
15	27	103 4	0 1	240					180	yes

* Required facilitation afforded by opening mouth

within the range of normal variation. The tests in the hot box, therefore, revealed no significant disturbance in temperature regulation. All of the cats reacted normally in the cold box and after 3 hours exposure to a temperature around 40°F. only one cat had a temperature as low as 99.8°F. These tests in the hot and cold box were as a rule made 2 weeks or more after the placing of the lesions, the exact number of days being indicated in the table.

As a final test the 6 cats of group B were decorticated under ether anesthesia 28 to 53 days after the last operation. During recovery from the anesthesia decorticate polypneic panting occurred in all but one (Cat 10). Another (Cat 14) panted only when the mouth was forced open. Stretching

the muscles of mastication apparently acts as a stimulus favoring panting (Kleyntjens, 1937). The failure of Cat 10 to show decorticate panting, although it did pant in response to heat, cannot be attributed to the lesion, for the area destroyed in this cat was also destroyed in Cats 13, 14 and 15 which did pant following decortication.

The small lesions in the cats of group A were placed at various positions in the thalamus damaging chiefly the medial nuclei but also involving some of the nuclei of the lateral group. In the first 3 cats of group B the lesions were intermediate in size between those in the last 3 of this group and those in the cats of group A. In Cats 10 and 12 the damage extended forward from the rostral border of the habenular nuclei. In Cat 11 the habenular nuclei were destroyed by the large lesion shown in Fig. 1, A and B, which extended laterally far enough to destroy the *centre médian* and damage the pars posterior of the lateral nucleus. It obliterated the caudal end of the third ventricle. Only the most ventral part of the zone of transition between the hypothalamus and the tegmentum of the mesencephalon was left intact.

By far the greatest damage was done in the last 3 cats of group B. In Cat 13 all of the medial portion of the thalamus was destroyed from the level of the anterodorsal nucleus backward to the border of the superior colliculus. The dorsomedial, habenular and parafascicular nuclei were destroyed bilaterally as were also the posterior commissure and the nucleus in the laterally spreading fibers of the posterior commissure, sometimes called the nucleus of the posterior commissure. All of the nuclei of the midline were either destroyed or greatly atrophied, and due to their disappearance and the absence of the dorsomedial nucleus the dorsal part of the third ventricle was greatly enlarged (Fig. 1, C). The walls of the third ventricle were badly damaged at the level at which it joins the cerebral aqueduct (Fig. 1, D).

In Cat 14 the damage began immediately behind the three anterior nuclei. There was bilateral destruction of the dorsomedial and other midline nuclei and also of the habenular and parafascicular nuclei. The nucleus in the laterally spreading fibers of the posterior commissure was destroyed on one side but intact on the other. The caudal part of the posterior commissure was intact. The lesion extended lateral-ward on both sides destroying the *centre médian* and damaging the pars posterior of the lateral nucleus. On one side it extended ventrally to the surface of the field H of Forel while on the other the nucleus sub-parafascicularis was intact.

In Cat 15 the damage extended forward to the anterior thalamic nuclei. The dorsomedial, parafascicular and habenular nuclei were destroyed bilaterally. The nucleus in the laterally spreading fibers of the posterior commissure was destroyed on one side, but almost intact on the other. The anterior half of the posterior commissure was destroyed. The parafascicular nucleus and the *centre médian* were destroyed bilaterally. The pars arcuata of the ventral nucleus and the pars posterior of the lateral nucleus were extensively damaged. The damaged area extended ventrally to within a short distance of the fields of Forel.

DISCUSSION

The medial nuclei of the thalamus which were completely or nearly completely destroyed in the last 3 cats are the ones most intimately associated with the hypothalamus and it would be to them that one would look for any control which the thalamus might exert over body temperature.
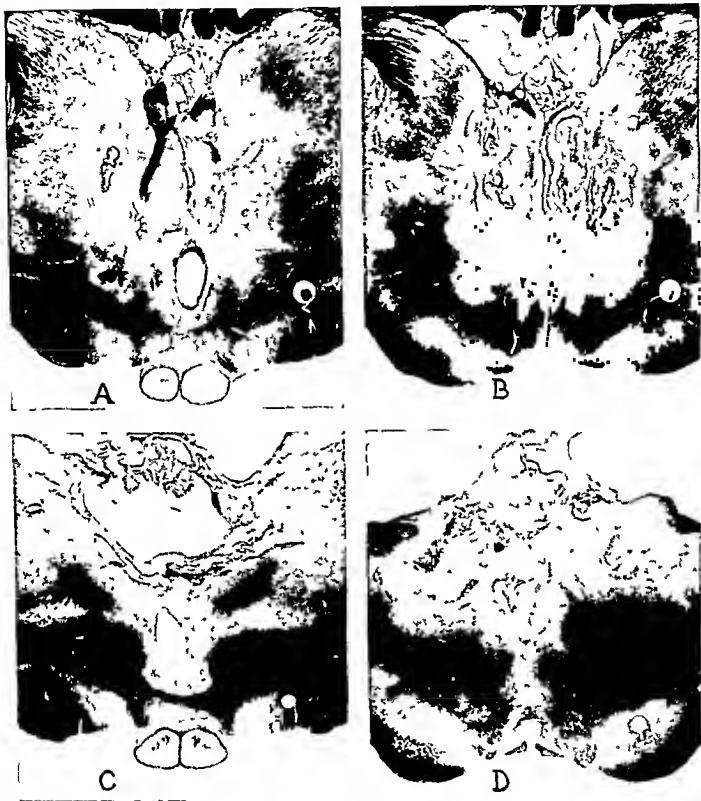
FIG 1 A and B represent photomicrographs from transverse sections of the brain of Cat 11 at the level of the lower border of the mammillary bodies (A) and at a level slightly caudal to the mammillary body (B) C and D represent photomicrographs from transverse sections of the brain of Cat 13 at the level of the mammillary body (C) and at the level of the third nerve (D)

The lateral nuclei which serve to relay impulses to the cerebral cortex would not be likely to be concerned in temperature regulation. Moreover decorticate cats in which the lateral thalamic nuclei had been removed or had undergone degeneration regulated body temperature in an apparently normal manner although they were more inclined to shiver than normal cats in a

cool environment (Pinkston, Bard and Rioch, 1934; Bard and Rioch, 1937). These decorticate cats panted when they became overheated.

There would seem, therefore, to be no reason to attribute any essential part of temperature regulation to the thalamus if it were not for the observations of Lilienthal and Otenasek (1937) who found that the polypneic panting, which occurs in acutely decorticate cats, was not abolished by removal of the hypothalamus to the level of the caudal border of the mammillary bodies so long as the caudodorsal part of the thalamus remained intact. Removal of this part of the thalamus abolished panting. On the basis of these observations they postulated the existence of a center for polypneic panting in "the caudodorsal portion of the thalamus, an area which lies below the habenular complex and surrounds the anterior part of the iter." But, since our Cats 11, 13, 14 and 15 in which this part of the thalamus was destroyed, panted in a normal manner in hot box tests and showed typical polypneic panting when decorticated, it cannot be said to contain a center which is essential for panting.

Special interest attaches to Cat 11 which as shown in Fig. 1 B had lesions that left only the most ventral part of the zone of transition between the hypothalamus and mesencephalic tegmentum intact. Farther forward the fields of Forel were destroyed but the region dorsolateral to the mammillary bodies was bilaterally intact (Fig. 1 A). The fact that this cat was not somnolent and showed no disturbance in temperature regulation is to be attributed to the integrity of these regions. It has been shown that lesions dorsolateral to the mammillary bodies cause somnolence in the monkey (Ranson, 1939), catalepsy in the cat (Ingram, Barris and Ranson, 1936) and marked disturbances in temperature regulation in the monkey (Ranson, Fisher and Ingram, 1937) and in the cat (Clark, Magoun and Ranson, 1939). The figures published in these papers show that the lesions were often not confined to the region dorsolateral to the mammillary bodies but extended dorsally into the fields of Forel and even into the thalamus proper. The results obtained on Cat 11 supplement those in the earlier experiments and show that these more dorsal parts may be destroyed without causing these symptoms. The absence of these symptoms in this cat is to be explained by the fact that it is through the region dorsolateral to the mammillary bodies that the chief part of the descending path from the hypothalamus runs (Ranson and Magoun, 1939).

SUMMARY

Damage to the thalamus causes no obvious disturbance of temperature regulation.

The caudodorsal portion of the thalamus does not form an essential part of the mechanism responsible for panting.

At the level of and just caudal to the mammillary bodies the descending paths from the hypothalamus which are concerned with temperature regulation lie near the ventral surface of the brain.

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EFFECTS OF FRONTAL LOBE LESIONS ON TEMPORALLY ORGANIZED BEHAVIOR IN MONKEYS*

JOHN L. FINAN

From the Department of Psychology and Laboratory of Physiology, Yale University, New Haven, and the Psychological Laboratory, St. John's College, Annapolis, Maryland

RECENT interpretations of frontal lobe function have made use of the concepts of "synthesis"[3] and "temporal organization" (serialization) of behavior,[19,25] and the evidence warrants recognition of some such principle of associative connection. Many agree, however, that further analysis will require fractionation of these descriptive concepts into experimentally workable and verifiable hypotheses. The investigations of Jacobsen[17-25] have served this end by contrasting performance of monkeys in the delayed response test, which is lost after bilateral ablation of the frontal areas, with that of simple discrimination learning, which is retained. Jacobsen points out that an essential difference between the two kinds of performance lies in the absence of differential sensory cues in the delayed response situation. The present investigation was designed to test in other situations the validity of this difference.

Two problems involving temporal discrimination have been studied. These tasks possess, in common with the delayed response tests, the absence of immediate stimulus cues to which the subjects can respond. The *first* problem involved use of an observation box divided by a falling door into two chambers equipped with "punishment" grilles. The animal, placed in a compartment, was forced by appropriately timed electrification of the two grilles, to remain for a fixed period of 10 sec. after the elevation of the door, and to cross into the opposite compartment during a subsequent 11 sec. interval (Fig. 1). The subject was thus trained to respond in the safe interval between the premature and tardy punishment intervals. The *second* technique employed a simple rectangular maze consisting of alternative pathways to food, different in the sole respect that one side entailed a longer time of enforced detention than the other (Fig. 2). Training on this problem was continued according to a method which eliminated nontemporal cues until the animals showed a stable preference for the side of shorter confinement.

Loss of temporal discrimination habits following operation would ally the behavioral and neural processes of these adaptations with those of delayed response performance, and indicate that the absence of differential cues is the responsible factor. Postoperative retention of these habits, on the other

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hand, by demonstrating that frontal lobe ablation affects delayed response and temporal discrimination habits selectively, would indicate the existence of separate behavioral processes mediated by independent neural mechanisms. In addition, the problem of whether or not "serialization," or the "temporal organization of behavior," may validly be regarded as the per-
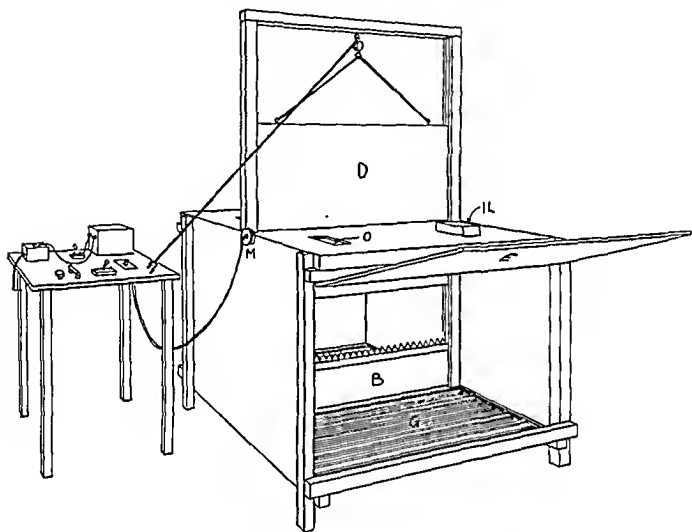
FIG. 1. Diagram of shuttle-box used in temporal discrimination training. The box (dimensions, 152×91×76 cm.) was divided into two equal compartments by a portcullis door (D) which rested, when lowered, on a sheet iron door-sill (B) 25 cm. in height. The floor was constructed of 3 mm. iron strips 12 mm. wide, and with a 30 mm. separation, alternately connected to form two independent circuits on either side of the central barrier (B). Both shock-systems were supplied with 110 V. 60 c./sec. current reduced by a 100,000 Ω variable resistor and a 24,000 Ω fixed resistor in series. Observation of the animal's behavior was accomplished by means of illumination (IL) and observation (O) apertures, the latter being covered with one-way vision glass. Manual controlling devices were mounted on a table-top adjacent to the apparatus.

formance mechanism lost after ablation of the frontal areas, would be critically answered.

EXPERIMENTAL METHODS AND SUBJECTS

Shuttle-box. An observation box was employed similar in principle to one used by F. L. Ruch[39] and by Dunlap and Gentry[5,11] in discrimination studies with white rats (Fig. 1). It was essentially a chamber divided into two compartments by a communicating doorway through which the animal could pass from one side to the other. The interior of the chamber offered no overhanging surfaces on which the animal might support itself to

escape shock. A current of approximately 0.001 A. was found adequate to meet the behavioral requirement of shock without unduly exciting the animals. Raising the door by means of a pulley system constituted the signal stimulus. Another stimulus beginning with the opening of the door was a weak tone obtained by impressing a 60 c./sec. 110 V. current across a speaker unit which fitted into the wall of the box in a central position.

To respond correctly in the shuttle-box, the animal must move from the initially occupied compartment to the opposite side during an 11 sec. interval beginning 10, sec. after the onset of the signal stimulus. A trial consisted of an interval of 35 sec. initiated by the opening of the portcullis door and the onset of tone. Lowering of the door together with the cessation of tone ended the trial. A correct response was scored when the animal arrived on the opposite grille not earlier than 10 sec. and not later than 20 sec. after the door was raised. Premature responses, earlier than 10 sec. were punished with shock on the opposite or "contralateral" grille, and failure to respond before the 20th second was punished by shock on the same or "ipsilateral" side. Response-latencies were measured with a stop-watch to the nearest fifth second, and a description of the motor-pattern of response was recorded. A work-session consisted usually of 20 trials.

The following *control* procedures were observed. Training was conducted as nearly as possible at the same time on successive days. A variable daily period of adaptation was allowed the animal upon introduction to the box. To insure the resumption of a quiescent posture in preparation for the next trial, trials were separated by short periods of time varied in duration to avoid conditioning to the inter-trial interval. It is unlikely that periodic or progressive sounds served as cues inasmuch as incidental noises which occurred in the environment were variable and did not appear to influence the behavior of the animals. Apparatus cues were made impossible by throwing the switch controlling the opposite grille only after the animal had responded. Intentional variation in the posture and movements of the experimenter did not alter the established response. An additional control of secondary cues is afforded by the fact that behavior remained unchanged when the controls were manipulated by other experimenters.

The establishment of the correct "shuttling" habit was accomplished in two stages. The simple habit of crossing to the opposite compartment in response to ipsilateral shock following the onset of the signal stimulus was first built up. Later training developed the correct timing of the response during the safe interval. During the preliminary training, door-tone stimulation was followed by the ipsilateral shock beginning at the 3rd sec. and continuing until the 20th sec. in the event the animal did not cross sooner. The onset of this shock was gradually dropped back from the 3rd to the 20th sec. When anticipatory crossing occurred with some regularity this mode of training was discontinued to avoid fixating the simple response which tended to creep forward in time to the signal stimulus. In the later training, the ipsilateral shock was activated from the 20th to 25th sec. the contralateral shock from the 1st to the 10th sec. Training was continued until the animals attained a stable level of approximately 80 per cent correct responses in 200 trials.

Temporal discrimination maze. The apparatus (Fig. 2) is an adaptation of a maze employed by Sams and Tolman[40] in discrimination studies in white rats. It consisted of two equidistant, alternative pathways to a food chamber with detention-doors allowing controlled confinement periods in the two pathways. The first of the routine five daily trials began with the raising of doors A and B and the unlocking and raising of door E. From the choice-point the animal was free to pass under either door, the chosen door being lowered immediately to form a closed detention-chamber with door C or D. The criterion of a choice consisted of the animal's entering a detention-chamber far enough to permit safe closure of the door. After the lapse of the 30 or 120 sec. time interval, door C or D was opened along with gate F admitting the animal to the food-chamber where it found a slice of banana. In preparation for the next trial, the entrance and food cages were then exchanged without removing the animal. To eliminate the possibility of response to a quicker return to the starting position after the correct response, cages were not intersubstituted until 4 min., including detention-time, had elapsed since the beginning of the trial. A control of secondary cues was provided by the fact that other observers were able to manage the apparatus without alteration in the subjects' performance.

Following an initial 24 hr. habituation period the preferred side was determined for each animal by five runs made with the two routes temporally equal at 30 sec. detention. The non-preferred side (chosen 0–1 times) was then selected to be the 30 sec. detention-

route, while the preferred side (chosen 4–5 times) was made the 120 sec pathway When the initial preference had been broken down and the temporally shorter route chosen with the frequency demanded by the criterion of 15 correct responses in 20 trials, the formerly shorter side was made the longer Under these reversed conditions training was continued until the criterion was met a second time For the animals operated before training, this point marked the end of the experiment The normal animals were, at this time, subjected to operation and subsequently brought to the previous degree of mastery with the detention intervals in the same right left relationship as before operation The relationships were again reversed and the animals forced to meet the criterion for the fourth time

Delayed response The delayed response control experiment served as a behavioral verification of the extent of lesions, as well as an additional proof that these particular animals were, in accordance with Jacobsen's general findings on animals without frontal areas, incapable of correct response in this situation For an exact account of the apparatus and method, the reader is referred to Jacobsen's description[20] Briefly, the animals were shown in which of two drawers a slice of banana was concealed, and after intervals varying from 0–30 sec a door was raised allowing the subject to leave a confinement chamber to
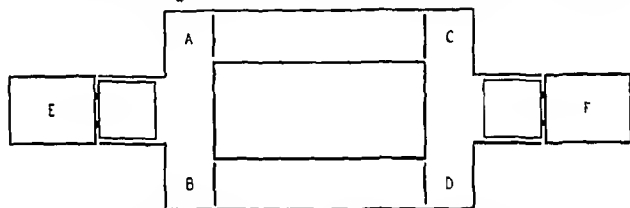
FIG 2 Floor of temporal discrimination maze With the exception of wire cages E and F, the maze was constructed of wood covered on the superior surface with hardware cloth The lengths of the various units were as follows entrance and exit alleys 30 cm , choice and exit chambers 120 cm , detention chambers 132 cm , the apparatus was uniformly 41 cm in height Doors (A, B, C and D) were controlled remotely by pulley ropes which terminated behind a one way vision screen at the front of the apparatus

choose between the drawers Following habituation to the apparatus, a series of test trials was run The reward was presented on the right or left in chance order Trials involving no delay were frequently interspersed among the test trials in order to avoid excessive frustration from repeated failure

Subjects Four immature mangabey monkeys (*Cercocebus torquatus atys*) served as subjects They were housed in large cages and fed on a standard laboratory diet Regular monthly weighings indicated, with one exception, that the animals maintained a normal state of health throughout employment Details concerning animals may be found in the appendix

Surgical and anatomical procedures The objective of the operations was the complete removal of the frontal association areas, shown by Jacobsen and collaborators[23] to be essential for delayed response performance This region includes the frontal pole and lies lateral to the frontal sulcus, extending posteriorly to the arcuate sulcus Actually the attempt was made to remove all tissue corresponding to Brodmann's[4] areas 9 10 11 12, sparing area 8 (eye fields) by extending the lesion along an imaginary line projected from the inferior limb of the arcuate sulcus to the longitudinal fissure Operations were carried out in both one and two stages * Aseptic precautions were observed throughout Tissue was incised with the Davis Bovie electrosurgical knife and removed by blunt dissection, whenever

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* The author is greatly indebted to Drs J F Fulton, C F Jacobsen and A E Walker for surgical assistance For a detailed description of the surgical procedures see Fulton and Keller [10]
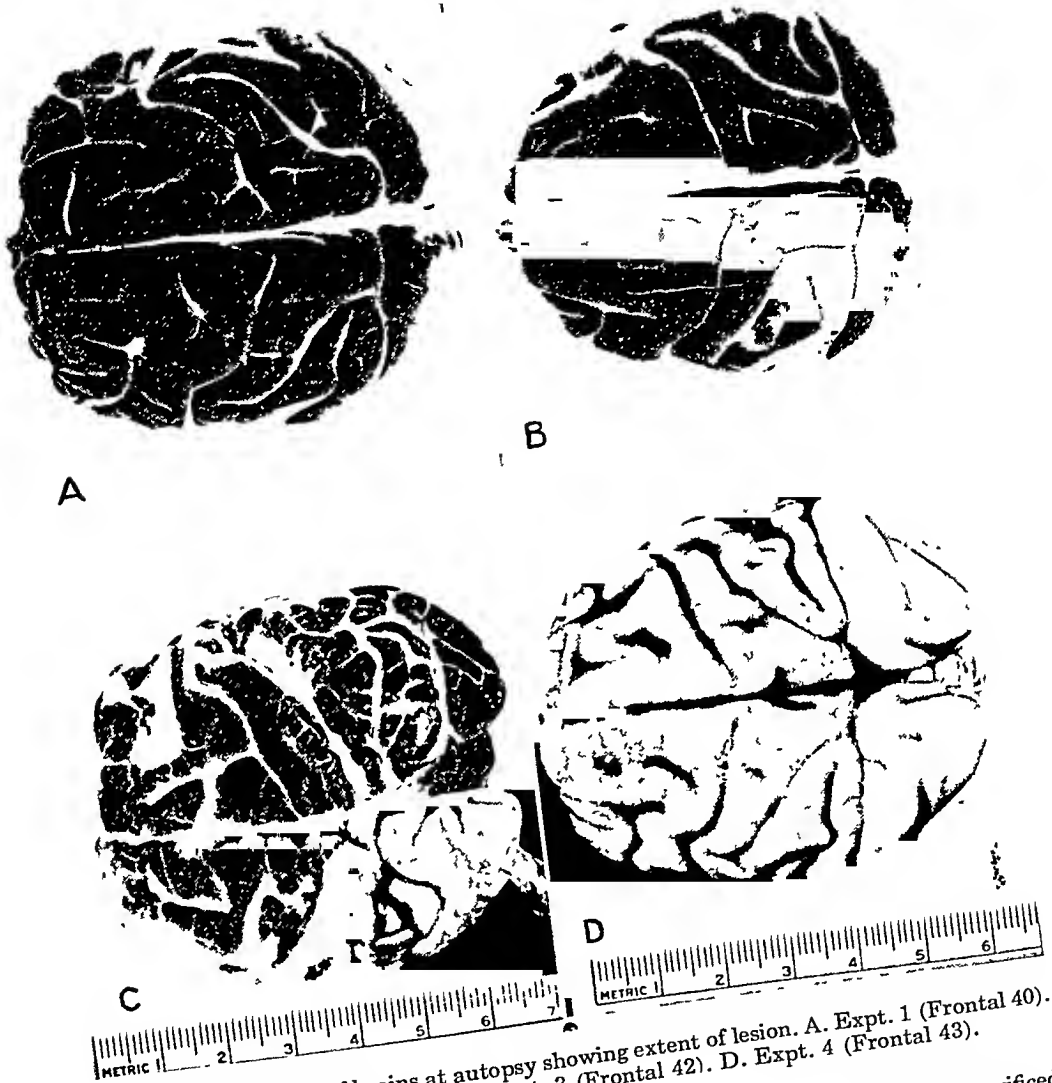
FIG. 3. Photographs of brains at autopsy showing extent of lesion. A. Expt. 1 (Frontal 40). B. Expt. 2 (Frontal 41). C. Expt. 3 (Frontal 42). D. Expt. 4 (Frontal 43).

possible in one piece. On completion of the postoperative tests, the animals were sacrificed and brought to autopsy. The brains were fixed by formalin injection. Lesions were reconstructed from three sources: (i) the drawing of the operative field and lesion traced on cellophane at the time of operation, (ii) histological examination of the extirpated block, and (iii) careful gross examination of the brain removed at autopsy.

RESULTS

Shuttle-box

Training period. Two animals, Experiments 1 and 2 (See Appendix), successfully mastered this problem to the 80 per cent criterion. The simple
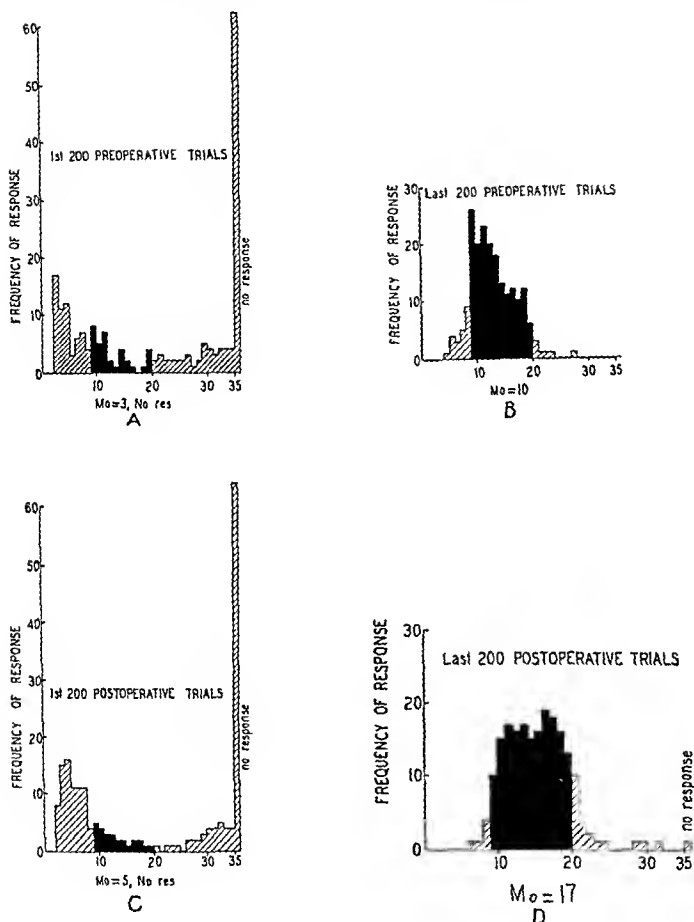
FIG. 4. (Expt. 1). Summary of results with shuttle-box. Frequency distribution of response-latencies for each sec. of the 35 sec. interval.

crossing habit, described in the procedure, was established in 14 trials in Experiment 1 and in 20 trials in Experiment 2. In the course of the following 49 trials of Experiment 1, and 20 trials of Experiment 2, the beginning of

the shock was dropped back gradually from the 3rd to the 20th second. In order to avoid fixation of a habit of premature response, the contralateral shock was then introduced. In spite of the fewer trials required in Experiment 2 in learning to cross, the resultant behavior under the double shock condition was the same for both subjects and consisted regularly of alternate anticipatory crossing and complete failure to respond. During the first 200
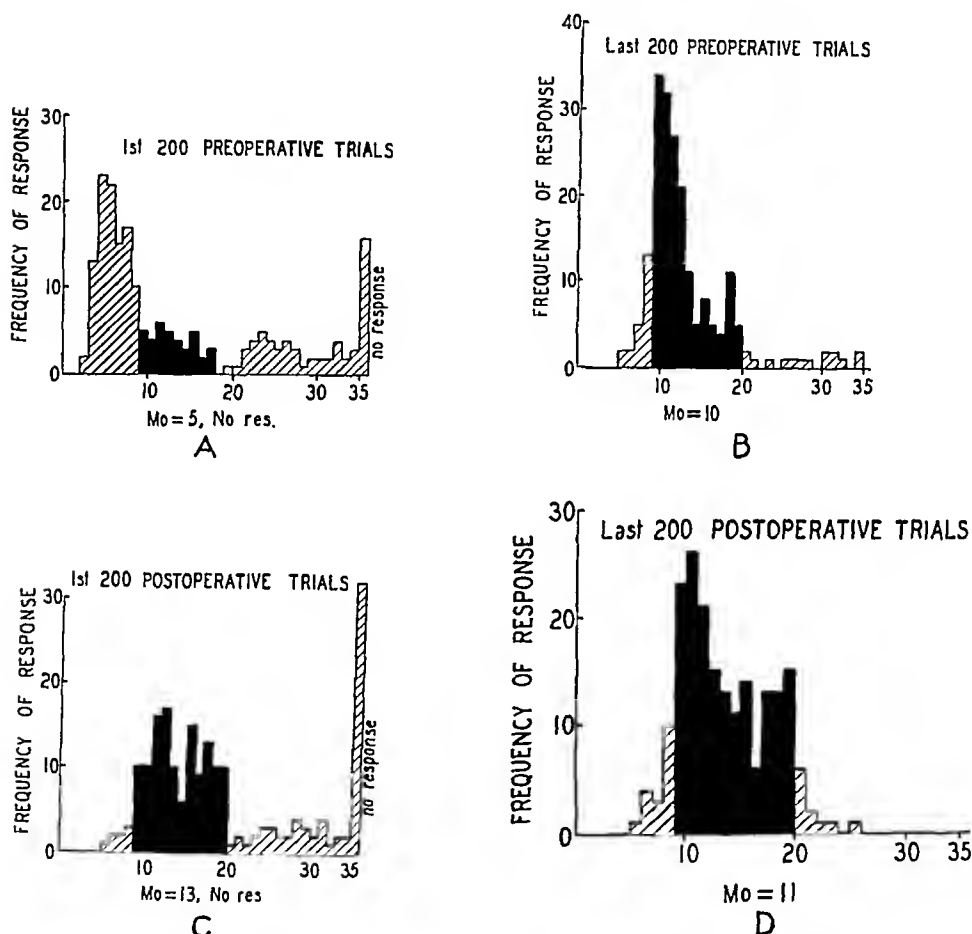
FIG. 5 (Expt. 2). Summary of results with shuttle-box. Frequency distribution of response-latencies for each sec. of the 35 sec. interval

trials, represented in Fig. 4A and 5A, modal responses for both animals fell into two groups; one with a central tendency early in the 35 sec. interval probably resulting from the initial short-delayed conditioning, the other with a maximum value at "no response" probably resulting from the introduction of contralateral shock. Both types of response appeared to depend on the immediately preceding errors. With continued training under the same conditions, the modal response assumed a position intermediate be-

tween these two extremes of anticipation and tardiness until, in the last 200 preoperative trials, both animals responded most frequently at the earliest safe point. Comparison of Fig. 4B and 5B demonstrate the consistency of response-latencies.

The course of acquisition of the habit is plotted for both animals in Fig. 6 in which each point of the curve represents the percentage of correct responses for successive hundreds of trials. The curve of acquisition for Experiment 1 indicates chance performance during the first 800 trials, rising sharply during the next 100 trials to maintain a stable level of slightly more than 80 per cent. The course of learning in Experiment 2 rides somewhat less
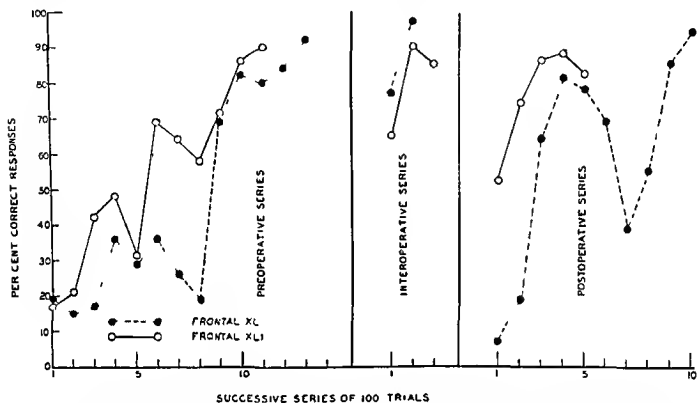
FIG. 6 (Expts. 1 and 2). Summary of preoperative and postoperative training in shuttle-box, showing percent correct responses for successive series of 100 trials.

sharply in roughly cumulative peaks until a stable level of 86 per cent correct responses was achieved. Behavior in the apparatus appeared to parallel the degree of mastery indicated by the quantifiable aspects of response. With the appearance of the correct habit, both animals substituted for the earlier habit of passive waiting, a marked activity which consisted of repeatedly approaching and retreating from the barrier, and circling around the chamber. These movements diminished perceptibly in frequency and amplitude with the immediately approaching safe-interval, and usually terminated in an abrupt halt at the barrier which was then crossed slowly and cautiously.

Interoperative retention. Figure 6, describing the course of retention after unilateral frontal lobectomy, indicates that the preoperative level of mastery was surpassed and averages of 87 and 88 per cent correct responses stably maintained for Experiments 1 and 2. Both animals tended to respond

3 sec. later in the safe interval than previously. In addition the distribution of responses during the entire safe interval was more equitable than in the earlier trials. Data obtained from the two animals were consistent in demonstrating that left unilateral ablation of the frontal areas caused no impairment in the accuracy of response to a temporal interval.

Postoperative retention. For 3 weeks following the second operation, Experiment 1 failed to show correct response to stimulation in the apparatus, while within a week postoperatively, Experiment 2 responded with a degree of accuracy equivalent to that of the preoperative criterion. The slump in performance in Experiment 1 immediately after operation (Fig. 6) can probably be considered the result of infection; first, because of the coincidence of the affliction and the impairment of the habit, and second, because of the absence of a similar effect in Experiment 2 which was uncomplicated.

A period of "no response" well marked in the immediate postoperative behavior of both animals (See Fig. 4C and 5C), was supplanted by a short period of anticipatory crossing similar to that of the original learning. A third phase was characterized by multiple crossing during a single trial; after responding correctly, the animals shuttled between compartments as many as two to seven times before the door was lowered ending the trial. Occurring immediately after the second operation with a frequency of nearly 100 per cent, the number of repeated crossings diminished with progressive training without ever dying out completely. No comparable behavior had been observed during the preoperative training of either animal.

The criterion was surpassed in Experiment 2 during the second and third hundred postoperative trials and was stably maintained. In Experiment 1 the attainment of 79 per cent correct responses for the fourth and fifth hundred postoperative trials was followed by a slump in performance. It will be seen from Fig. 6 that the criterion was met postoperatively a second time during the 9th and 10th hundred postoperative trials. This inversion in the retention curve illustrates a type of variability well known in operated animals. A similar phenomenon has been reported in conditioning studies on normal human subjects[14]. The latencies of the last 200 responses, described in Fig. 4C (Experiment 1), were scattered with a slight preponderance of crossings late in the safe-interval. Figure 5D (Experiment 2) shows that the modal response fell somewhat earlier with a less equitable distribution of responses over the safe-interval. The general tendency for correct responses to occur at any point within the safe-interval correlates roughly with progressive training in both subjects.

Prior to the operation, the typical mode of response during the period of mastery consisted of several circlings around the box, terminated by a rapid succession of waverings close to the barrier. The complete inactivity immediately following frontal lobectomy was supplanted by a violent activity. During the last of the postoperative training, however, *movements* were almost entirely confined to the region of the barrier, where the animal picked at the grids, screws and other exposed parts of the apparatus. Move-

ment, then, in accordance with the findings of Wendt,[43] tended to vary in locus and amplitude, remaining fairly constant in frequency throughout training.

In summary, seriatim bilateral extirpation of the frontal areas in the two monkeys was not followed by permanent loss of ability to respond correctly to a temporal interval as presented in the shuttle-box problem.

Temporal discrimination maze. Four animals were used in this problem. Experiments 3 and 4 included preoperative training with later testing for postoperative retention. In Experiments 1 and 2 training in the problem for the first time was conducted after operation. Following 5 preference trials the problem in Experiments 3 and 4 was mastered to the criterion of 15/20 trials. The detention ratio of the two sides was then reversed and the reversal learned to the same degree of mastery. A quantitative account of the training appears in Table 1. Motivation was consistently higher in Experiment 4 than in Experiment 3, although with continued daily training, the maze was run in the latter with a minimum of dallying at the entrance and choice-points.

Table 1 *Summary of preoperative and postoperative results on the temporal discrimination maze showing the number of trials and errors in each series to reach a criterion of 15/20 trials correct (criterion trials included)*

Training series	Frontal 42		Frontal 43		Frontal 40		Frontal 41	
	Trials	Errors	Trials	Errors	Trials	Errors	Trials	Errors
PREOPERATIVE								
First series	91	52	50	21				
Reversal series	41	26	109	78				
POSTOPERATIVE								
First series	17	2	16	1	25	9	65	34
Reversal series	39	20	30	13	70	44	35	15

In the postoperative testing series, the completeness of retention was demonstrated by the absence of errors (Table 1). The possibility that merely a position-habit had been retained rather than the capacity to respond differentially to temporal intervals was controlled by running a subsequent reversal series which was mastered in Experiments 3 and 4 as indicated in Table 1. The fact that during the postoperative retention series in Experiment 4 the food-reward was invariably refused, emphasizes "escape" over the food motive in the solution of this problem. Resultant differences in the manner of response manifested in increased loitering and inactivity at the choice of exit points, in no way altered the accuracy of this animal's performance. The problem-attitude and manner of solution in Experiment 3 appeared to be unaffected by operation. In Experiments 1 and 2 the counter-preference series was mastered to the criterion, following which the same norm was reached under reversed conditions (Table 1). It merits mention that the operated animals learned this problem with somewhat greater ease than the normal animals.

Delayed response

Three animals served as subjects. Experiment 4 was run preoperatively with delay intervals ranging between 0 and 20 sec. The percentage of correct responses at each interval, presented in Table 2, show that this subject was entirely capable of correct choice within the limits tested. Postoperative retention tests on the same animal yielded results in substantial agreement with those reported by Jacobsen.[20] The interposition of even the smallest delays resulted in failure to respond with better than chance scores, although visually oriented response involving no delay was entirely possible (0-delay trials, Table 2). Continued daily tests allowing optimal opportunity for correct response showed no improvement in the performance of this task. Errors were variable and showed no consistent tendencies other than transitory position habits. The subject was frequently distracted and often retreated to the rear of the cage, refusing to work.

Table 2. Summary of the preoperative and postoperative training on delayed response.

Experiment	Period of Delay in Seconds					
	0	2	5	10	20	30
NO. 4. PREOPERATIVE TESTS						
No. of trials	71	3	2	76	24	11
Per cent correct	91	100	100	92	83	100
NO. 4A. POSTOPERATIVE TESTS						
No. of trials	166	11	119	87		
Per cent correct	92	37	58	53		
NO. 1. POSTOPERATIVE TESTS						
No. of trials	369	183	244			
Per cent correct	83	52	58			
NO. 2. POSTOPERATIVE TESTS						
No. of trials	187	90				
Per cent correct	83	49				

The remaining two animals were trained in delayed-response for the first time after operation. (In Expt. 1, 796, and in Expt. 2, 277 trials were run.) Table 2 indicates that both subjects were unable to master the delayed response problem after bilateral removal of the frontal areas. When visual cues were allowed (0 sec. delay), a high level of correct response was obtained in Experiment 1. With delays of 2 and 5 sec. results were only slightly above chance. On the trials involving no delay for Experiment 2 response was well above a chance level, dropping to 50 per cent when a 2 sec. delay was employed. Attempted solutions were extremely variable and seemed to manifest no systematic tendencies other than the occasional position preferences already described for Experiment 4.

Summary.—Results using 4 monkeys on 2 different temporal discrimination problems agree in indicating that neither unilateral nor bilateral lesions

of the frontal association areas cause impairment in the capacity to learn or retain the habit of responding correctly to temporal intervals. In contrast with these habits, the delayed response performance was permanently impaired by the same cortical injuries. The results presented here thus contain two kinds of evidence relevant to the effects of lesions of the frontal association areas on complex adaptive habits of a superficially related order and complexity.

Interpretation

The complex behavioral changes resulting from bilateral frontal injury in human patients cannot easily be forced into any single conceptual formula. The hypotheses of "association,"[2] "synthesis,"[3] "temporal organization,"[19,25] "active inhibition"[1,16] and "facilitation"[12] represent independent attempts to unify the variable symptoms of the frontal lobe syndrome, all falling short of scientific analysis because of their failure to define critical experimental alternatives. When, however, methodological differences are weighed, and adequate account taken of the sources of clinical error, the sum of both clinical and experimental evidence suggests that the situations which disclose frontal lobe deficit are predominantly those in which external stimulus control is at a minimum. Goldstein[12] reports a patient who was perfectly capable of rearranging small sticks into a roof-top angle a half minute after initial presentation, but who failed when required to reproduce the same angle pointing upward. According to Goldstein's analysis of this difference in performance, correct response was possible in the first instance because the stimulus relationships were apprehended as a *concrete* item of the patient's past experience, whereas, in the second case, it was necessary that the sticks be arranged as representative of a more abstract situation not encountered in previous experience. Summarizing the symptomatology of frontal lobe lesions, Goldstein notes that:

"(the patient) is incapable of recollection when he is asked to recall things that have nothing to do with the given situation. But when it is possible to put him into a situation to which the material inquired for belongs, recollection appears suddenly . . . he is able to learn new facts; he may be able to learn numbers, syllables, or movements by heart; he is able to hold in memory situations, facts of environment, etc., but he is able to reproduce these only in the same situation in which he has learned them. . . . Therefore the patient's performance consistently varies according as the task is embedded in a concrete or abstract situation" (12, pp. 36–37).

The interpretation of Brickner in terms of "synthesis" and that of Penfield and Evans[18] as a loss of capacity for "planned administration," are compatible with Goldstein's emphasis on the non-sensory character of those situations which reveal loss of adaptive function in cases of frontal lobe involvement.

The absence of differential cues proves to be the factor common to the more exact experimental situations which demonstrate behavioral changes in animals after ablation of the frontal areas. The following tests employed by Jacobsen to demonstrate frontal lobe deficit all show the same lack of

external stimulus support: (i) Instrumentation tests[25] in which a chimpanzee is presented with food and a stick necessary for drawing it in at opposite ends of a cage. Here the elements of correct solution may not be united in a single perception, but must be supplemented either by memory of the stick, or of the food on the unseen platform. (ii) Serial reaction studies[25] involving the possibility of two types of errors, namely those of serial order (anticipation) in which spatial cues were available, and those of direction of movement, in which they were absent. The learning of the sequence of response was much more rapid than that of appropriate direction. Response was markedly less successful when dependent on the inward or outward movement of a particular peg mediately associated with food. (iii) The delayed response technique[19,20,23] requiring differential response after enforced postponement, likewise eliminated differential sensory cues, since the paired aspects of the situation lend no clue to the correct response at the moment of choice. (iv) The delayed alternation situation[24] in which the absence of differential cues is expressed in the fact that correct response can occur only with reference to the immediately preceding response. The impaired performance obtained in these four situations stands in sharp contrast with the excellent retention or learning of problem boxes and discrimination problems in which the cues necessary to correct response are part of the immediate test situation.

On the basis of this evidence, Jacobsen has proposed that: "The peculiar contribution of the frontal association areas (appears to be) . . . the recall of a particular past event which may be only in mediate association with some aspect of the present environment, and the integration of recalled elements with the organism's stable habit systems" (20, pp. 55–56). As employed by Jacobsen, the concept of immediate memory implies a distinction, based on the dependence of certain types of performance on differential stimulus cues, between "retentive" or "associative" memory, and the fundamentally different kind of organization operative at the level of the memory span.

In terms of the principles outlined above, it will be noted that the shuttle-box problem requires response to an "absolute" interval of time, *without external stimulus support*, after self-enforced postponement of response. The temporal discrimination maze demands a "relative" discrimination of alternative detention intervals *mediately associated* with briefer detention. These tests have in common with the delayed response test these essential features: response after the lapse of an interval of time, and *the absence of differential cues in or directly related to the test situations*.

While the temporal situations here employed are similar to the delayed response test in the absence of differential cues, the two kinds of problem are distinguishable in terms of their differential susceptibility to the effects of cumulative training. Assuming with Nissen that a fundamental difference between delayed response and discrimination learning lies in the fact that in the former "the animal is not trained to the correct response by making

it . . . but instead must respond on the basis of a single unrewarded and unpunished presentation" (24, p. 132), we can describe the distinctive features which enable the temporal and delayed response habits to be differently affected by cortical ablation in terms of habit-acquisition. Waiving possible genetic differences between the two types of behavior in favor of a description in terms of performance on a given trial, we are forced, since the essential characteristics of both hinge on the *absence* of differential external cues, to seek differences in their underlying central processes. An analysis of these differences awaits a more precise investigation of the behavioral and neural processes operative in the two kinds of solution. The present research thus provides new lines for the fractionation of the learning process into conditioned response fixation and non-trial and error solution previously proposed by Lashley,[29,30] Jacobsen,[20] Maier,[33,35] Krechevsky[27] and Harlow[13] on neurological grounds, and by Köhler[26] and Tolman[42] on the basis of behavioral evidence. Our results delimit the concept of immediate memory further by showing the necessity for qualifying Jacobsen's definition in terms of the absence of differential stimulus cues.

A related purpose of the investigation was to determine the possible role of the "trace" reflex as the mechanism of delayed reaction. To assume in advance that the two behavioral processes may be identified, lends the problem a clear and simple experimental formulation and is further justified by the theoretical expectation that the trace component in many complex habit sequences provides a basic unity on which the serial order of the action depends.[15,31,41] Results from the two temporal discrimination problems, however, clearly show that neither retention nor relearning of the "trace" reflex type is dependent on the integrity of the frontal areas. In the light of these findings, the "trace" reflex as an integrative mechanism of serial habit-formation does not appear to represent a unitary function and hence requires restatement in terms of the conditions under which it operates together with the exact properties which distinguish it from other types of organization.

In a similar connection, Jacobsen has suggested that in so far as such concepts of frontal lobe function as "serialization" and "synthesis" are tenable, they are reducible to immediate memory or to the "temporal organization of behavior" defined operationally in terms of the delayed response situation. He writes: "It would thus appear that bilateral lesions of the frontal areas seriously impaired adjustment to situations involving temporal organization of behavior. On the other hand, ability to manipulate several sticks as tools was evident when the materials were presented in a spatially organized field and did not require sustained temporal organization" (25, p. 3.) As Jacobsen[20] later points out it is questionable whether this formulation, although experimentally more useful than the broader notions mentioned above, does not likewise lack value as a tool of rigorous scientific analysis. From our results it appears that temporal organization as tested in the present experiments plays no essential role in delayed response per-

formance and hence cannot, without further qualification, be considered the mechanism of immediate memory.

Speculation concerning the differences in the behavioral and neural processes underlying the two types of performance distinguished as association (retention) and non-trial and error solution (immediate memory) has been kept at a minimum in the absence of more complete evidence. Our results, however, demonstrate that the problem of further fractionating the learning process is genuine rather than conceptual. An attempt has been made to outline some of the experimental variables necessarily to be encountered in future attempts at its solution.

SUMMARY

The effects of removal of the frontal association areas of the cerebral cortex were studied in four monkeys with three types of behavioral tests, alike in the respect that differential cues were absent from the external situation, but different in respect of the degree of influence exercised by repeated training in the establishment of their corresponding habits. The tests were: (i) a temporal discrimination maze in which available sensory cues were mediately associated with the briefer detention of the correct pathway, (ii) a shuttle-box which required response to be made within a fixed interval after the onset of a signal stimulus, and in which, again, the cues necessary to correct performance must be supplied by the subject itself; (iii) the delayed response test in which the cues representative of immediately previous stimulation also had to be contributed by the animal.

The results are summarized as follows: *Unilateral* lesions of the frontal areas caused no impairment in performance on any of the three tests. *Bilateral* ablation of the frontal areas affected performance in different ways. (i) The ability to perform in a shuttle-box was retained without impairment. (ii) Retention of the maze test was not impaired, nor was the capacity to learn this task for the first time reduced. (iii) The capacity to respond correctly in the delayed response test was totally and permanently lost by the same subjects.

It is concluded that, although the frontal areas are essential to one type of centrally controlled behavior, these regions are not essential to other types of equally "representative" behavior investigated in this study. The concept of immediate memory is accordingly further qualified: (i) in terms of "associative" and "non-trial and error" learning, and (ii) in terms of discrete neural mechanisms underlying the two kinds of behavior.

APPENDIX

The similarity of methods employed and the consistency of the results obtained allow individual protocols to be presented in brief form. Clinical records of these animals are published elsewhere[36],[37].

Experiment 1—Bilateral ablation of frontal areas in two stages, retention of shuttle-box habit, abolition of delayed reaction, postoperative learning of temporal discrimination maze habit (Frontal 40)

The subject of this experiment was an immature female sooty mangabey (*Cercocebus torquatus atys*) weighing 1700 g The animal had been observed for several months prior to training and was readily adapted to work Its *preoperative training* (Dec 2, 1935 to Mar 9, 1936) was directed solely to the mastery of the shuttle box

First operation—Ablation of left frontal areas (*March 9, 1936*) A suitable anesthesia was obtained with Nembutal administered intraperitoneally A generous bone flap was turned down exposing the left hemisphere The dura was reflected and an attempt made to remove all tissue anterior to an imaginary line projected from the inferior arcuate sulcus to the mid line This included areas 8, and 9-10-11-12 of Brodman, area 6 was not encroached upon The weight of the freshly extirpated block was 1 84 g *Recovery* was uncomplicated by sensory or motor disturbances except for a transient object vision "hemianopsia" observed on the second postoperative day, involving the left temporal and right nasal fields Circus movements toward the side of the lesion were noticed immediately following operation, passing away on the third day Healing of the wound and the general state of health satisfactory

Interoperative training (*Mar 10-17*) Daily trials on shuttle box were begun on the first day following operation and were continued for seven days The preoperative level of mastery was maintained and even surpassed

Second operation—Ablation of right frontal areas (*Mar 21, 1936*) Under sodium amytal anesthesia the lesion made in the first operation was closely duplicated on the right side without alteration of the operative procedure The extirpated block weighed 2 7 g in the fresh state Immediate *postoperative recovery* was complicated by a superficial scalp infection observed on the second day following operation For the ensuing three weeks, the animal was depressed and without appetite During this period no alterations in sensory or motor function were observed With the recovery from infection, the animal became brighter, activity was more marked and the appetite strikingly increased over the normal preoperative level

Postoperative training (*Mar 27-Dec 7, 1936*) Testing on the shuttle box was resumed immediately after operation Following a period of "no response" the animal regained its previous level of mastery During the fifth postoperative month, delayed reaction training was undertaken but the animal was unable to master the problem Later, in the ninth month, training was conducted in the temporal discrimination maze habit, which the animal learned readily

Verification of lesions The animal was sacrificed on April 12, 1937, 13 months after the first operation The lesions (Fig 3A) include the regions of the frontal lobe known to be essential for delayed reaction performance The cortex corresponding to Brodmann's area 9-10 11-12 was completely ablated except for a few shreds of tissue on the extreme posterior surface of area 9

Experiment 2—Bilateral ablation of the frontal areas in two stages, retention of shuttle box habit, abolition of delayed reaction, postoperative learning of temporal discrimination maze (Frontal 41)

The subject of this experiment was an immature female sooty mangabey (*Cercocebus torquatus atys*), weighing 2900 g The animal had been observed for several months prior to training and was readily handled but remained excitable Its *preoperative training* consisted of daily trials in the shuttle box until the criterion of mastery was attained

First operation—Ablation of left frontal areas (May 12, 1936) Under sodium amytal anesthesia, all tissue anterior to the inferior limb of the arcuate sulcus and its imaginary extension to the midline was removed The weight of the freshly removed block was 2 6 g *Recovery* was uncomplicated By the first postoperative day appetite had returned and movements were executed normally, although activity was well marked Neither reflex nor sensory impairment was detected and nothing in the animal's behavior after the third day suggested any abnormality

Interoperative training (*May 13-June 2, 1936*) Daily test trials in the shuttle box conducted during this interval showed that the preoperative level of performance was maintained

Second operation (*June 2, 1936*) The right frontal areas were ablated following the

same procedure as before. The extirpated block weighed 2.2 g. *Recovery* was uneventful with no sensory or motor impairment. Hyperactivity and morbid hunger were first noticed on the fourth postoperative day and marked the entire postoperative course.

Postoperative training (June 3, 1936–Jan. 12, 1937). Testing on the shuttle-box was resumed on the first day and continued during the following three months, during which period it was shown that the habit had been retained. In the fifth postoperative month training on the delayed response was undertaken but the animal failed utterly to learn this problem. Training on the temporal discrimination maze was given seven months after operation and this habit was acquired efficiently. All work with this animal was interrupted shortly thereafter by the onset of sexual swelling and menstruation accompanied by marked behavioral changes.

Verification of lesions. The animal was sacrificed April 12, 1937, ten months after operation. Autopsy examination showed that the cortex anterior to the arcuate sulcus had been entirely destroyed in both hemispheres with the exception of a few mm. of tissue on the mesial surface which remained intact (Fig. 3B). This spared tissue, however, is posterior to the region essential to delayed reaction. Area 6 (premotor area) and the basal ganglia were undamaged.

Experiment 3—Simultaneous bilateral ablation of the frontal areas; retention of the temporal discrimination maze habit (Frontal 42).

The subject of this experiment, an immature female sooty mangabey (*Cercocebus torquatus atys*), weighing 2000 g. was exceptionally timid, but tractable and easily handled. *Preoperative* training was concerned exclusively with the temporal discrimination maze.

Operation—Ablation of frontal areas of both sides (Nov. 13, 1936). An attempt was made to remove in one piece, all tissue anterior to areas 6 and 8 in the left hemisphere. The extirpated block weighed 2.4 g. in the fresh state. A similar but slightly larger lesion (2.6 g.) involving the corresponding regions was made in the right hemisphere. On the second *postoperative* day some restriction of the conjugate movement of the eyes to the right was observed. There was no perceptible impairment of posture or locomotion. No hyperactivity was noted, although the appetite was apparently increased beginning the fourth day following operation. The wound healed by first intention.

Postoperative training (Nov. 15–Dec. 6, 1936). Beginning 2 days after operation, routine testing on the temporal discrimination maze was resumed which showed that the habit had been retained.

Verification of lesions. On the 30th day after operation, the animal died following a second operation involving the parietal areas. The extent of the lesions of the frontal areas made in the first operation is shown in Fig. 3C. (A second lesion in the parietal lobe also visible in the Fig. 3C is irrelevant to the present consideration.) All tissue anterior to the inferior limb of the arcuate sulcus excepting a few mm. immediately anterior, identified as area 8, had been removed. The posterior limits of the lesions were equivalent on the two sides and corresponded to the posterior boundary of Brodmann's area 9.

Experiment 4—Simultaneous bilateral ablation of frontal areas; retention of temporal discrimination maze habit, abolition of delayed response.

The subject of this experiment was an immature male mangabey (*Cercocebus torquatus atys*), weighing 4000 g. The animal was under observation for several months before experimentation, and proved cooperative and easily handled. Prior to operation the animal was trained in the temporal discrimination maze and in the delayed response apparatus.

Operation—Ablation of both frontal areas (Nov. 19, 1936). Under sodium amytal anesthesia, all tissue anterior to Brodmann's area 6, save the eye-fields, was removed from the left hemisphere in one block weighing 2.1 g. The corresponding regions on the right side were similarly removed, the block weighing 2.0 g. During *recovery* there were no sensory or motor disturbances, except for some restriction of ocular movement on the second day. The onset of pulmonary tuberculosis (verified at autopsy) at this time presumably accounted for the animal's loss of appetite and initiative.

Postoperative training (Nov. 21–Dec. 24, 1936). Testing on the temporal maze was instituted on the second day following operation and the animal maintained his previous level of mastery of this habit. During the period from the 15th to 34th days, testing on the delayed response problem was conducted but the ability to respond correctly of this test had not been retained.

Verification of lesions. The animal was sacrificed on the 56th postoperative day. The

approximate boundaries of the lesion are visible in Fig 3D In both hemispheres, all tissue identifiable as areas 9 10 11 12 had been removed save for 1 mm of badly traumatized tissue on the lateral aspect of area 9 No injury had been caused to the basal ganglia the ventricles had been spared, and area 6 appeared undamaged The posterior limit of the lesion was well in back of the region shown by Jacobsen, Haslerud and Taylor to mediate delayed response

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RELATION OF STIMULATION TIME OF RECEPTORS TO RECOVERY TIME IN THE NERVOUS SYSTEM: VISUAL, OLFACTORY AND AUDITORY SENSES*

CHARLES A. ELSBERG AND H SPOTNITZ
Clinical Research Laboratories of the Neurological Institute of New York

IN OUR studies of the special senses of vision, smell and hearing, investigations were made of the relationship between the time of action of a specific stimulus, and the time required for recovery from the stimulus. The results of these investigations appear to demonstrate that there is a simple mathematical relationship between stimulation time and recovery time. While the peripheral receptors must play an important part,—photochemical in vision, physical or physicochemical in smell and hearing,—the speed of recovery (*i.e.*, of the return to equilibrium) appears to be based upon a neural factor common to all of the special senses that were investigated.

The principles on which the tests of olfaction and of vision are based, and the apparatus used and procedures followed, have been described in detail in papers published previously For the tests of vision, the minimum intensity of light required to see a test object was first ascertained The eye or eyes were then exposed for increasing lengths of time to a brighter light of known intensity The time required after each light adaptation, until the individual could again recognize the test object illuminated by threshold light, was the time required for recovery (i e, the time required for dark adaptation)

For the olfactory tests, the minimum volume of odor required for the recognition of an odorous substance was first ascertained The olfactory membrane was then exposed for increasing lengths of time to a stream of air and odor of a definite volume rate, and in each test the time required until the individual could again recognize the odor at its threshold value (the minimum identifiable odor, or MIO) was the time required for recovery from olfactory fatigue (olfactory adaptation) The apparatus used and the methods for testing hearing will be described in detail in another report They are based on a similar principle to that on which the olfactory and visual tests are based For our present purpose, the following facts may be mentioned The apparatus used was the audiometer 6A made by the Western Electric Company, by which the wave frequency can be varied up to 8190, and the intensity can be varied between 0 and 120 decibels The sound was conveyed to the ears by double ear-phones which were allowed to remain in place during each series of tests—no matter whether one or both ears were stimulated

The threshold for a tone of any frequency was first ascertained The threshold was the minimum intensity which could be recognized when one or both ears were exposed to the tone for periods of one second at intervals of one second The ear was then exposed to a greater intensity of the same tone† for increasing lengths of time, and then to the threshold intensity which had previously been determined, until the tone was recognized In each test, the time that elapsed from the conclusion of the more intense stimulation to the time when the threshold stimulus was again recognized was the period required for recovery (the duration of auditory fatigue or the time required for auditory adaptation).

TESTS OF THE VISUAL SENSE

A large number of tests of foveal vision with red light (beyond 610$\mu\mu$ of the spectrum) showed that there is a simple mathematical relation between

† With the apparatus at present available, it is impossible to reproduce exactly the same tone at a higher as at the lower intensity (see Stevens and Davis, 1938, pp 72–73) The differences are, however, so small that they may be disregarded

the time during which the eye was exposed to the light stimulus (light adaptation) and the time required for dark adaptation (*i.e.*, the time which elapsed before a threshold stimulus could again be recognized as such). It was found that for each intensity of light the square root of the recovery time divided by the cube root of stimulation time had a constant value, or

$$\frac{\sqrt{\text{Recovery Time}}}{\sqrt[3]{\text{Stimulating Time}}} = C, \text{ or } \frac{RT^{1/2}}{StT^{1/3}} = C, \text{ or } \frac{RT}{StT^{2/3}} = C.$$

This relationship between stimulation time and recovery time is shown, for example, by the following series of tests for light intensities of 40 and 80 foot candles (at a distance of 40.5 cm. from the eyes).

Table 1. *Test object with contrast. Total area 6400 sq. mm., white area 1600 sq. mm , black area 4800 sq. mm. Intensity of illumination of test object 5.4 foot candles at a distance of 3.75 m. Intensity of bright light used for light adaptation 40 foot candles Duration of exposure to bright light 5, 10, 15, 20, 25 and 30 sec. Averages of 3-5 tests.*

Time of Stimula-tion	Time of Dark Adap-tation	Time of Stimulation$^{2/3}$	Time of Dark Adaptation — Stimulation Time$^{2/3}$	Calculated Time of Dark Adaptation	
				Based on $C = 2.63$	Based on average of $C = 2.67$
sec.	sec.			sec.	sec.
5	7 9	2 92	2 63	7 7	7 8
10	11.9	4 64	2 56	12 2	12 4
15	16 1	6 1	2 64	16 04	16 3
20	19 9	7 4	2 68	19 5	19.8
25	22 6	8 6	2 63	22 6	23 0
30	24 7	9 7	2 55	25 5	25 9

Table 2. *Test object with contrast. Total area 6400 sq. mm. Intensity of illumination of test object 4.96 foot candles; intensity of light used for light adaptation 80 foot candles. Duration of exposure to bright light 30, 60, 90 and 120 sec. Average of 3-4 tests.*

Time of Stimulation	Time of Dark Adaptation	Stimulation Time$^{2/3}$	Time of Dark Adaptation — Stimulation Time$^{2/3}$	Calculated Time of Dark Adaptation Based on Average of $C = 29.9$
sec.	sec.			sec.
30	27 5	9 7	2 83	29 .
60	47 6	15 4	3 09	46 04
90	60 4	20.0	3 02	59 8
120	77 7	24 5	3 02	73 3

TESTS OF THE OLFACTORY SENSE

From the following illustrative tests, it is demonstrated that for the sense of smell, the relation between stimulation time and recovery time for odor used, is the same as that for vision, namely,

$$\frac{RT}{StT^{2/3}} = C$$

Table 3 Coffee odor, unilateral tests of right nasal passage, MIO 8, stream injection of odor 2000 cc per min

Duration of Stream Injection	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time − Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 12 9
sec		sec		sec
10	4 64	60	12 9	59 9
20	7 4	90	12 2	95 5
30	9 7	120	12 4	125 1
60	15 4	210	13 6	198 6

Table 4 Coffee odor, unilateral tests of right nasal passage, MIO 8, stream injection of odor 4000 cc per min

Duration of Stream Injection	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time − Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 30 8
sec		sec		sec
5	2 92	90	30 8	89 9
15	6 1	180	29 5	187 9
25	8 7	270	31 0	267 9

Table 5 Citral odor, unilateral tests of right nasal passage, MIO 7, stream injections of odor 2000 cc per min

Duration of Stream Injection	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time − Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 28 3
sec		sec		sec
10	4 64	120	25 8	120 0
20	7 4	210	28 3	190 9
30	9 7	270	27 8	250 3

Table 6 Citral odor, unilateral test of right nasal passage, MIO 8, stream injections of odor 1000 cc per min

Duration of Stream Injection	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time − Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 11 0
sec		sec		sec
20	7 4	60	8 11	81 4
30	9 7	120	12 3	106 7
60	15 4	180	11 7	169 4
120	24 5	270	11 0	269 5

Tests of the Auditory Sense

As shown by the series of tests in Tables 7, 8 and 9, the relation between stimulation time and recovery time for the auditory sense was the same as that for the visual and olfactory senses, namely

$$\frac{RT}{StT^{2/3}} = C$$

for each frequency used in the tests.

Table 7. Binaural tests at threshold; intensity of stimulation 92 decibels. Frequency 1024. Tests at 15-second intervals. Averages of 3 to 5 tests.

Stimulation Time Injection	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time ÷ Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 1.2
sec.		sec.		sec.
5	2.92	4	1.36	4.50
10	4.64	5	1.19	5.6
15	6.1	7.5	1.23	7.3
20	7.4	9.1	1.23	8.9
30	9.7	11.9	1.21	11.6

Table 8. Left ear tested at threshold; intensity of stimulation 90 decibels. Frequency 1024. Tests at 15-second intervals. Averages of 3 to 5 tests.

Stimulation Time	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time ÷ Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 1.74
sec.		sec.		sec.
5	2.92	5.1	1.74	5.8
10	4.64	8.2	1.76	8.1
15	6.1	10.8	1.76	10.6
20	7.4	12.5	1.70	12.9
30	9.7	18.0	1.84	16.9

Table 9. Left ear tested at threshold; intensity of stimulation 80 decibels. Frequency 2048. Tests at 15-second intervals. Averages of 3 to 5 tests.

Stimulation Time	Stimulation Time$^{2/3}$	Recovery Time	Recovery Time ÷ Stimulation Time$^{2/3}$	Recovery Time Calculated on Basis of 2.7
sec.		sec.		sec.
5	2.92	7.9	2.7	7.9
10	4.64	12.0	2.58	12.5
15	6.1	16.2	2.65	16.5
20	7.4	18.4	2.49	19.9
30	9.7	25.2	2.6	26.2

DISCUSSION

It is well known that excessive stimulation of a peripheral receptor can lead to alterations in the structure and function of that receptor, which may take many hours for complete recovery. In our investigations of recovery time only moderate stimuli were utilized. These were based upon the principle clearly expressed by Froehlich that the central nervous system fatigues for comparatively weak stimuli according to the principles of relative fatigue. It was found that the difference in intensity between the fatiguing stimulus and the threshold stimulus determined the maximum recovery time. As the difference between the two intensities increased, the recovery time was lengthened; as the difference decreased, the recovery time became shortened. Thus the limits within which the stimulation time formula held was determined in part by the difference in intensity between the moderate stimulus and the threshold stimulus.

There were two other limiting factors for the recovery time formula: (i) the effect of stimulation time was limited. For the sense of smell it was observed that the recovery period was not lengthened when the stimulation time was longer than 3 min. For the senses of vision and hearing there was usually no significant increase in the recovery time when the stimulation time was longer than one and one-half minutes. (ii) Under normal conditions, the maximum duration of the recovery period of the sense of smell was usually about 7–9 minutes; for the sense of vision, the maximum recovery period was about 80 sec., and for the sense of hearing about 40 sec.

The formula: Stimulation Time$^{2/3}$ ÷ Recovery Time = Constant enabled one to predict the recovery time only when the duration of the stimulation time was less than the maximum effective stimulation time and only when the duration of the recovery time was less than the maximum recovery time obtainable with the stimulus.

In our investigations of the literature we have been unable to find any studies concerning the quantitative relation between stimulation time and recovery time in which the *intensity* of stimulation was kept constant but the *time* of stimulation was varied. The fact that recovery time was proportional to the $\frac{2}{3}$ power of the stimulation time was referred to in a paper by one of the writers (Mills Memorial Lecture). In that paper we recorded the fact that a large series of tests of foveal vision with red light showed that, if the test object and the intensity of light by which it is illuminated are kept constant, the formula for dark adaptation could be expressed as:

$$TDA \div (I^{1/3} \times StT)^{2/3} = C^*.$$

From this equation it was possible to suspect that if the intensity of the stimulating light was kept the same *i.e.*, if in the above equation I is a constant and is given any value (say 1) then

$$TDA \div StT^{2/3} = C$$

* TDA = Time required for Dark Adaptation; I = Intensity of light used for light adaptation; St T = Stimulation Time; C = Constant.

or the time required for dark adaptation (time of recovery) divided by the $\frac{2}{3}$ power of the time of stimulation should have a constant value. In other words, the recovery time should be proportional to the $\frac{2}{3}$ power of the stimulation time. In the tests described in this report, the visual experiments were actually made with a constant intensity of stimulation and the relationship between stimulating time and recovery time was found to be that which had been predicated from our previous tests.

That the same simple mathematical relationship between stimulating time and recovery time was also found in tests of the olfactory and the auditory senses appears to indicate that this relationship may be a general one. In the present state of knowledge, such a generalization may be only suspected until the time when based on the same principle, quantitative tests of the sense of taste and of the various modalities of somatic sensation have been carried out or have become possible. In the tests of the visual sense, the relationship between stimulating time and recovery time held only when the entire peripheral receptor (retina) was stimulated. If the experiments were so arranged that only one-half of the retina was stimulated by the bright light (*i.e.*, was light adapted), then the relationship was altered.

In a previous report, we have made the suggestion that this $\frac{2}{3}$ power might indicate that the recovery time depends upon a surface reaction. The evidence which appears to make it probable that this surface reaction occurs in the nervous system and not in the peripheral receptor apparatus has been summarized in a paper already published. There were some indications in our tests that a changed mathematical relation between the reaction of the peripheral receptors and the reaction of the nervous system may result under special conditions. As yet the significance of these alterations is not at all clear.

The mathematical relationship between stimulation time and recovery time suggested in this report, is in all probability only an approximation, but by means of this relationship it has been possible usually to predict (within the error of the experiments) the results of other tests from one experiment.

SUMMARY

1. For the special senses of vision, smell and hearing, there is the same simple mathematical relationship between the duration of stimulation of the peripheral receptors and the duration of the recovery period.

2. This relationship indicates that the duration of the recovery period is proportional to the $\frac{2}{3}$ power of the duration of stimulation.

3. This relationship is limited by certain factors.

4. The relationship may be based upon a neural factor common to all of the senses that were investigated.

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ANATOMY, PHYSIOLOGY AND SURGICAL CONSIDERATIONS OF THE SPINAL TRACT OF THE TRIGEMINAL NERVE*

A. EARL WALKER

*From the Division of Neurology and Neurosurgery,
University of Chicago Clinics*

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INTRODUCTION

A PRIME essential for the success of surgical intervention to relieve trigeminal pain is a knowledge of the finer anatomical and physiological relationships of the fifth cranial nerve. Neurosurgeons, realizing this fact, have in large measure been responsible for the careful experimental, clinical and anatomical studies of the fiber arrangement of the trigeminal root. Recently, for the relief of facial pain, several operative procedures on the central trigeminal pathways[7,15] have been suggested, the most promising being section of its descending tract[16]. A study of the anatomy and physiology of the spinal tract of the trigeminal nerve then becomes increasingly pertinent.

Central terminations of the fifth nerve

Although embryologically the fifth cranial nerve is homologous to a spinal nerve, its central relationships are quite different. In the human embryo His[12] noted that the "root pieces" of the trigeminal nerve arise out of bundles with a crossed course. Windle[23] has confirmed this observation in the cat embryo in which the fibers from the anteriorly developing ophthalmic division of the ganglion enter posteriorly and somewhat medial to those from the mandibular and maxillary divisions. With further development and curvature of the brain stem, the entrance zone of the ophthalmic division comes to lie in the inferior and medial portion of the root. In support of this theory Davis and Haven[6] state that in cats partial section of the root, as practiced in human beings, produces a keratitis, and anaesthesia of the forehead and maxilla.

This arrangement of the root of the fifth nerve has been questioned by Sjöqvist[16] on the basis of his fiber analysis. He found that the fibers with a diameter of 4μ or less, to which presumably belong those conveying pain and temperature, are more numerous in the ophthalmic division of the trigeminal nerve and in the upper part of the sensory root throughout its extent. He concluded, therefore, that there was no rotation of the root to such a high degree as supposed by Windle[23] and Davis and Haven.[6] He did not find the small fibers arranged peripherally as did Windle in the cat or cen-

trally as Davis and Haven[6] found in man. While Davis and Haven[6] state that the portion cut in Dandy's suboccipital operation on the trigeminal root is the ophthalmic division, Sjöqvist[16] maintains that it is the maxillo-mandibular group, and states that the sensory changes present are found about the mouth or lower lip. Although Dandy[5] does not admit consistent sensory alterations following this operation, most writers support Sjöqvist's contention. In Bailey's experience[1] the perioral region shows sensory changes. In two such cases Van Wagenen[19] found disturbances in sensation around the mouth.

The arrangement of the fibers passing into the pons has been studied in the newborn mouse by Cajal.[4] The majority bifurcate, one branch passing superiorly and one down the tract of the spinal root of the trigeminal nerve. Cajal,[4] however, stated that he could not be sure that all fibers bifurcated. Later investigators have laid little stress on the non-bifurcating fibers, although the majority admit their presence. In 1923 Gerard[10] studied the entering fibers of the fifth nerve on pyridine silver preparations of the cat's brain. She believed that the majority divided, but admitted that she saw no bifurcation of small fibers, either myelinated or unmyelinated. Windle[22] studied the brain stems of newborn mice and foetal pigs impregnated by Golgi silver method. He describes three types of fibers: (i) Heavy fibers which divide dichotomously in a typical T or Y shape, one branch ascending to the main sensory nucleus and the other going inferiorly to the spinal nucleus of the fifth nerve. (ii) Some fine fibers bifurcate, but the majority turn downward at the peripheral margin of the bifurcating fibers to pass into the spinal root of the trigeminal nerve without branching. (iii) A few fibers, usually of large size, turn upward to enter the main sensory nucleus without giving off a descending branch. The following were the proportions of these fibers:

Bifurcating fibers	51.34 per cent
Descending non-bifurcating	42.25 per cent
Ascending non-bifurcating	6.41 per cent

The topical arrangement of the fibers in the spinal tract of the fifth nerve seems to be fairly well agreed upon by all investigators. Bergman[2] showed that when the sensory root was completely degenerated at its entrance into the pons, the spinal tract was likewise fully degenerated. After experimental lesions of the root, Bergman[2] noted that those animals having an absent corneal reflex and marked keratitis had degeneration in the ventral part of the spinal tract. When the corneal reflex was preserved and the keratitis absent only the dorsal portion of the tract was degenerated. In 1901 Spiller and Frazier[18] confirmed this localization and in 1904 Wallenberg,[21] and in 1907 Kutner and Kramer[13] independently, came to the same conclusion. The last believed, however, that the ophthalmic division extended to the lowest level in the spinal tract of the fifth nerve, the maxillary

fibers did not reach so far and the mandibular fibers passed down a still shorter distance.

The significance of this arrangement of the fibers of the spinal tract of the trigeminal nerve has been indicated by several previous writers. Both Winkler[24] and Spiller[17] have suggested that the fibers which pass down the spinal tract are concerned in the appreciation of pain and temperature, while those which terminate in the main sensory nucleus are carrying touch and proprioception. Later writers have confirmed this finding on both clinical and experimental grounds. Gerard[10] concluded from an analysis of clinical cases of thrombosis of the posterior inferior cerebellar artery that the impulses carrying pain and thermal sensibilities from the fifth nerve descend through the bulb in the spinal tract of that nerve, and pass at various levels to the cells of the spinal nucleus; whereas the tactile impulses from the face pass to the cells of the main sensory nucleus. Her studies following experimental section of the spinal trigeminal root in the bulb of the cat pointed to the same conclusion.

Sjöqvist[16] in a recent study of pain conduction in the trigeminal nerve has utilized the findings of Gasser and Erlanger[8] that finely myelinated fibers carried pain and temperature sensibilities. Sjöqvist's[15] analysis (expressed as percentage of total fibers) of the fibers of the spinal tract of the macaque monkey which he stated was similar to that of man, is as follows:

	Less than 3μ	Less than 4μ
Level of the superior olive	71.8	85.0
Just above inferior olive	77.0	89.4
Caudal part of the inferior olive	80.9	91.9

Because this tract contains such a large percentage of small fibers he concludes that it must be concerned with the passage of pain and thermal impulses. However, in an apparently similar analysis carried out by Gerard*[10] (on the cat, but said to be comparable to man) there was a quite different ratio of small and large fibers in the spinal tract of the trigeminal nerve.

	Less than 3.7μ	Less than 4.8μ
Level of the superior olive	53.5	72.2
Just above inferior olive	48.2	74.6
Caudal part of the inferior olive	39.5**	69.6**

Moreover, although she states that the large fibers decrease in the lower levels of the trigeminal tract, her figures do not show that the relative proportion of smaller fibers is increased.

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* The statistics given here are based on the average of the figures given by Gerard for 3 or 4 cases. The figures for the individual cases, however, did not vary greatly.

** This figure is probably slightly too small, because Gerard does not give the number of myelinated fibers measuring $0-3.7\,\mu$ at the lower level of the inferior olive. The calculations are based upon her figure for the fibers of this size at the bifurcation of the pyramids.

One must therefore conclude that although there is a large proportion of small fibers in the descending tract of the trigeminal nerve there are also larger fibers, whose size is comparable with that of the fibers known to carry touch and proprioception

Clinical significance. The role of the spinal tract of the trigeminal nerve has been determined largely by clinical studies of dysfunction resulting from vascular lesions of the lateral portion of the medulla oblongata which is irrigated by the posterior inferior cerebellar artery. Thrombosis of this vessel produces a characteristic syndrome, one element of which is usually said to be a dissociated anaesthesia on the ipsilateral side of the face. This is considered, and probably correctly so, the result of involvement of the spinal root of the trigeminal nerve. Practically all authors state that after this type of vascular insult there is a marked analgesia on the ipsilateral side of the face with preservation of tactile sensibility.

OBSERVATIONS

Two typical cases of this syndrome, both of the left posterior inferior cerebellar artery, have been carefully examined for the sensory changes on the face, neck and throat. Both cases had a left Horner's syndrome, sensory disturbances over the left side of the face, no masseter, pterygoid or facial weakness on either side, a hemihypalgesia on the right side below the face and incoordination of the left arm and leg. Both were examined at least one month after the ictus at which time they appeared to be reliable witnesses.

Method of examination All examinations were carried out with the eyes closed or the patient blindfolded Using Von Frey's hairs and pins, representative areas about 4 sq cm in size were stimulated at irregular times with one test object for ten times The sequence was interrupted two or three times to stimulate some other area of the face to be certain that the attention was maintained The series of ten stimulations usually took about one minute A short rest was allowed between series, and if the patient showed any signs of fatigue, which readily manifested themselves by inconstant replies and inattention, the examination was terminated, and continued another day The results are given as the number of stimuli correctly appreciated in a series of ten tests On the whole the results obtained by these graduated series of stimuli were quite consistent

CASE 1 (HBF) The following were the results of graded stimulation of the face.

Pain	Forehead		Cheek		Chin	
Grams	R	L	R	L	R	L
0 125	0	0	0	0	0	0
0 5	0	0	0	0	0	0
0 75	6	0	5	0	0	0
1 0	6	1	3	0	7	0
2 0	8	0	9	0	6	0
4 0	10	2	10	3	10	0
6 0		0		0		0
8 0		0		0		0

If, however, the stimuli were rapidly repeated near the same point the patient complained bitterly of the pain, which was rather diffusely localized to the point stimulated The pain of such stimulation was much more severe on the affected side than on the normal

Touch	Forehead		Cheek		Chin	
G./mm.	R.	L.	R.	L.	R.	L.
0.5	1	0	2	0	2	
1.0	7	1	5	1	3	4
2.0	8	0	7	3	7	3
3.0	7	1	10	2	7	3
4.0	9	1	10	6	10	8
5.0	7	6	10	3	9	9
7.5	7	7	10	7	9	9
10.0	10	7	10	10	10	10
15.0		10				

CASE 2. (HH). Examination six months after thrombosis of the left posterior inferior cerebellar artery.

Touch	Forehead		Cheek		Chin	
G./mm.	R.	L.	R.	L.	R.	L.
0.5	1	0	7	0	3	0
1.0	7	0	6	0	7	0
2.0	9	0	9	1	9	2
3.0	10	0	10	3	10	4
4.0	10	0	10	3	10	4
5.0	10	2	10	4	10	4
7.5	10	4	10	5	10	6
10.0	10	5	10	7	10	7
15.0	10	8	10	10	10	10
Pain						
Gm.						
0.125	0	0	0	0	0	0
0.5	3	0	3	0	1	0
0.75	9	0	9	0	9	0
1.0	10	0	10	0	10	0
2.0		0		0		0
4.0		0		1		1
6.0		1		3		2
8.0		1		2		2

Heat and cold are lost in the same distribution on the face as pin prick. The tongue, soft palate and pharynx on the left side are analgesic. The patient says that he has a constant burning pain in the left side of the face.

Comment. These cases lack the conclusiveness afforded by anatomical verification of the lesion. The clinical findings would suggest, however, that the softenings were largely confined to the medulla oblongata, and did not involve pontine structures; for there was no weakness of the facial, masseter, pterygoid or external rectus muscles. It seems likely, then, that the main sensory nucleus of the fifth nerve was not involved by the softening. One may, therefore, assume that the sensory disturbances in the face were due to interruption of the descending tract of the fifth nerve. It then follows that the latter carries fibers which are mainly, but not exclusively, concerned

in the transmission of pain and temperature sensibilities. Tactile impulses are also carried in this tract.

Trigeminal tractotomy

The recently devised operation of trigeminal tractotomy offers an opportunity to study the sensory defects following a lesion practically confined to the descending root of the fifth nerve. In this operation the spinal tract of the trigeminal nerve is sectioned with a sharp scalpel at the level of the middle of the inferior olive (Fig. 1).
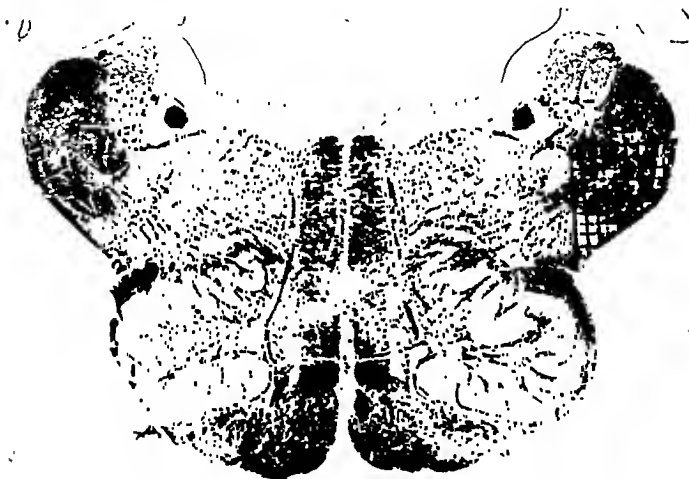
FIG. 1. Section through the bulb at approximately the middle of the inferior olivary nucleus to show the position of the descending root of the trigeminal nerve and (crosshatched) the extent of the incision in the operation of trigeminal tractotomy. (Kulschitsky myelin stain ×6.)

Sjöqvist[16], who devised the operation, suggested it as an alternative to section of the trigeminal root in the posterior fossa. Such a procedure might have certain advantages over the classical retrogasserian neurectomy. (1) Since touch is largely preserved the disagreeable numbness which frequently follows retrogasserian neurectomy might be avoided (2). Keratitis might be less likely to develop (3). It could be performed in an individual in whom the temporal route was inadvisable owing to local neoplastic in-

volvement or infection (4). Facial and masseter paralysis could be more easily avoided. Whether all these advantages will be realized remains to be determined for as yet only 11 cases* have been reported, 9 by Sjöqvist[16] and 2 in this paper. That dysesthesias will be eliminated by this procedure seems unlikely in view of the fact that patients with posterior inferior cere-
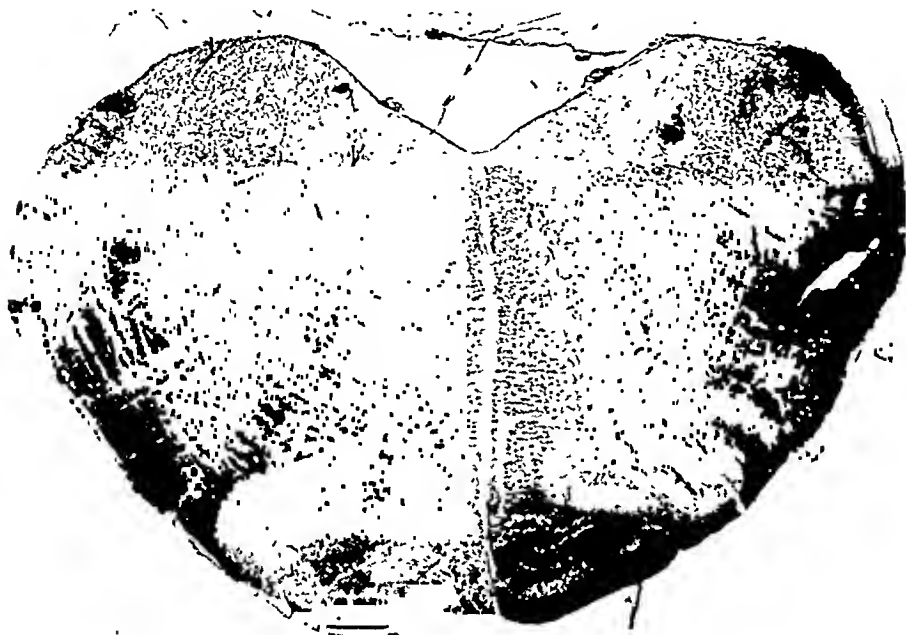
FIG. 2. Section through the bulb at the level of the inferior third of the inferior olivary nucleus to show the actual section of the descending root of the trigeminal root in the macaque monkey. (Marchi preparation ×6.) (M-4-38, Bl. viii, 2.)

bellar thrombosis frequently complain of a burning pain in the ipsilateral side of the face.

But there are certain obvious disadvantages to this operation. (i) The operative field is in the posterior fossa, where bleeding is frequently difficult to control without grave consequences, and from whence one cannot retreat so readily as from the middle fossa. (ii) It is difficult to be sure that enough of the tract has been cut. In two of Sjöqvist's patients no sensory disturbances could be demonstrated postoperatively, and in one of the present cases the first division fibers were spared, rendering the operation only

* Since writing this paper Rowbotham (ROWBOTHAM, G. F.: Treatment of pain in the face by intramedullary tractotomy. *Brit. med. J.* 1938, 2: 1073–1076) has reported 3 additional successful cases, and Jackson and Ironsides (JACKSON H., and IRONSIDES, R. Left trigeminal pain treated by Sjöqvist's medullary trigeminal tractotomy. *Proc. R. Soc. Med.* (Sect. Neurol.), 1938, 32: 219–220) have added a further case.

partially successful. (iii) In three of Sjöqvist's cases involvement of the vagus root gave unilateral laryngeal paralysis. Although temporary in one case, the voice remained husky in other cases. (iv) Although no fatalities are reported to date, I doubt that the operation if used routinely, even in the hands of the most experienced neurosurgeons, would carry as low a mortality as the classical retrogasserian neurectomy.

For this reason the operation is not suggested as a routine procedure, but only to be used in carefully selected cases. It should be particularly valuable for the relief of pain in the neck and face from neoplastic lesions. In these cases it is frequently desirable to section the upper dorsal posterior roots as well as the sensory fibers from the face. Tractotomy in these cases is a much simpler procedure than section of the trigeminal root as it enters the pons.

Results of trigeminal tractomy. The operation was first tried on three macaque monkeys (Fig. 2). Immediately after operation the corneal reflex was constantly abolished and the animal did not respond to pin prick over the ipsilateral side of the face, although it did react to tickling of the ipsilateral nares. There was no weakness of the masseter muscles. In one animal there was a mild Horner's syndrome on the ipsilateral side (Fig. 3). The animals ran, jumped and climbed the day following oper-

FIG. 3. Photograph of a macaque monkey (M-4-38) to show the results of section of the descending root of the trigeminal nerve. The absence of the corneal reflex and Horner's syndrome on the right side are apparent. The lesion in this case is shown in Fig. 2. (Exposure in this and the following illustration was 0.5 sec.)

ation without the slightest unsteadiness or abnormality in their gait. Encouraged by these results and by Sjöqvist's report, two patients have had this operation performed for the relief of trigeminal neuralgia.

CASE 3. (TE), A housewife, age 44, was first seen in the University of Chicago Clinics on April 11, 1939 . . . . arp pain in the left side of the face for the past year, a . . . . years and a dead feeling in the right arm for a year. S· . . . . began to feel heavy and dull. As this progressed, walking became difficult. Three years later the right hand became numb, but improved after extraction of some teeth. A few months before admission the right hand again became numb so that she was unable to hold objects in it. At times when trying to walk she became dizzy. Two years ago she had her first attack of pain in the left side of the face. It did not recur until the fall of 1937 and then lasted only a short time. About 2 weeks previous to admission it returned, occurring 2 to 3 times a day. She described the

attacks as shocks radiating from the left cheek and mandible to the midline, but never above the eye. The paroxysms of pain lasted about 10 min. and were relieved by cold packs. They were initiated by pressure on the lower teeth, washing the face, eating, etc., so that the patient was afraid to brush her teeth or wash her face. Inquiries into her systemic functions, family and personal history, were irrelevant.

Physical examination was essentially negative aside from the neurological findings. She was well-oriented, but mentally dull and rather slow in cooperating. Her cranial nerves were normal, except for a vertical nystagmus on upward gaze, and markedly diminished corneal reflexes, especially the left which was practically absent. No sensory disturbances to pin prick or cotton could be determined over the face. Audiometer tests showed a mild nerve type deafness but the caloric reactions were normal.

There was marked weakness and spasticity of the right extremities and left leg. On finger-to-nose and heel-to-knee tests a severe intention tremor developed on the right side. Rapidly alternating movements were poorly performed on the right but fairly well carried out on the left side. *Sensory* tests were difficult to evaluate owing to the patient's mental torpor. There appeared to be a marked diminution to touch, pin prick, and temperature on the right leg below the knee. Proprioception was markedly impaired in the right fingers and toes. Vibration was also impaired in the right ankle, patella, and wrist, and slightly at these points on the left side. Objects placed in the right hand were frequently not even appreciated. The *tendon reflexes* were very lively and both plantar responses were extensor. Hoffmann's sign was positive on the right side. Examination of the spinal fluid showed no abnomalities. The patient was considered to be suffering from multiple sclerosis *with symptomatic tic douloureux.* Because of the marked sensory changes the possibility of an extensive intramedullary spinal cord tumor was suggested. To confirm the diagnosis and at the same time relieve the pain a trigeminal tractotomy was proposed to the patient who was quite agreeable to the procedure.

On April 16, 1938, the left spinal tract of the trigeminal nerve was sectioned under nembutal and local anaesthesia. A grayish translucent area was seen in

FIG. 4. *Case 3.* To show the absence of the corneal reflex on the left side following section of the descending root of the trigeminal nerve.

FIG. 5. *Case 3.* To show the boundaries of the sensory disturbances on the left side of the face. The line indicates the limits of hypalgesia and hypothermaesthesia, the inferior interrupted line the lower limit of tactile disturbance. Below the faint line at the level of the eye the disturbances were much more severe than above.

the lower portion of the bulb, presumably a plaque of multiple sclerosis

The patient had an uneventful recovery from the operation except for a pleuritis lasting 3 days Following the operation the patient was quite free from pain, and much more alert and active then formerly The neurological findings following operation were essentially the same as previously with the exception of the sensory status of the left side of the face (Fig 4)

Sensory examination On April 28, 1938 the sensory disturbances over the face were mapped for touch, pain, heat and cold, the boundaries marked with ink, and then the patient photographed The area of marked impairment for pain extended over the entire left side of the face, along the lower border of the mandible, across the masseter, in the auricle, and then vertically for 3 to 4 cm when it again extended posteriorly to the operative scar Below the level of the eye the loss was much greater than above that level, although the changes were quite definite even on the forehead. The boundaries for cold and heat coincided with those for pain Touch was impaired over a slightly larger area than pain In no place was there a complete loss for any . . . distinguish heat and cold, and the head from the . . . *corneal reflex* was sluggish, the left absent The m . . . There was no facial weakness (Fig 4)

Strength of hair	Chin		Cheek		Forehead	
Touch	R	L	R	L	R	L
0 5 g/mm	7	3	3	0		
1 0 "	9	6	5	2		
2 0 "	10	9	7	3		
3 0 "	10	7	10	2		
4 0 "	10	10	10	4	10	2
5 0 "	10	10	10	5		
10 0 "				7		
15 0 "			10	9		
Pin Prick						
0 125 g	0	0	0	0		
0 5 "	5	0	4	0		
0 75 "	8	0	8	0		0
1 0 "	9	2	9	4		5
2 0 "	10	6	10	8		10
4 0 "	10	9	10	9		

Post operative Sensation tested by von Frey's hairs

The patient returned *November 4, 1938*, for a check-up She had had no pain in the face and had felt much improved over her general preoperative condition Her legs were quite stiff, but she managed to get about with aid, and could use her hands sufficiently to cook and look after the house Her neurological examination was essentially the same as that immediately after operation The right corneal reflex was fairly active, the left absent Heat, cold and pinprick were appreciated on both sides of the face, but definitely less so on the left side in about the same degree and distribution as immediately postoperatively Deep pressure pain was markedly decreased on the left side Two point discrimination was slightly decreased over the left cheek Touch was said to be impaired in the same area as pain and temperature Localization of touch on the left side of the face was also slightly defective as compared to the right side The left side of the tongue and palate were hypaesthetic as compared to the right The uvula lay slightly to the left of the midline at rest and deviated to the right on phonation There was no dysphagia or dysarthria Graduated sensory examination of the face is shown in table at top of next page

CASE 4 (JS) A well-preserved male, age 50, was first seen in the University of Chicago Clinics on June 21, 1929, complaining of severe, paroxysmal, sharp, stinging pain in the right lower jaw during the previous 15 years Six years before admission an alcohol injection (the exact site is unknown) gave complete relief for two and one-half years Since that time the pain had been recurring at intervals particularly in the cold weather It was

Strength of hair	Chin		Cheek		Forehead	
Touch	R.	L.	R.	L.	R.	L.
0.5 g./mm.	3	0	3	3	1	1
1.0	3	0	7	2	1	0
2.0	9	1	7	3	3	3
3.0	10	5	10	6	7	1
4.0	9	2	9	4	6	3
Pin prick						
0.125	0	0	0	0	0	0
0.5	3	0	2	0	4	0
0.75	6	0	7	1	7	1
1.0	6	0	8	3	9	3
2.0	7	2	8	2	6	2
4.0	8	4	9	2	9	7
6.0	9	7	10	7	10	8

initiated by eating, drafts, shaving, etc. Recently two alcohol injections failed to give relief from the pain.

His personal and family histories were irrelevant.

The patient was in excellent physical condition. The only abnormalities found on neurological examination were a slight right lower facial weakness and an area of anaesthesia along the lower right jaw.

On June 22, 1929, a right retrogasserian neurectomy was performed by Dr. Percival Bailey, the lower two-thirds of the posterior root being sectioned and the motor division spared. There resulted a complete anaesthesia of the third division and a partial anaesthesia of the second but no motor weakness of the masseter or pterygoid muscles.

About a year after the first operation he began to experience attacks of pain in the left mandibular region occurring at irregular intervals. During the last year the pain had radiated into the cheek. About three weeks before admission shooting pains were noted in the left forehead. These pains were paroxysmal and characteristically initiated by cold drafts striking the face, touching the forehead, etc. At the time of hospital admission the more severe pain was present in the forehead. Anaesthesia was present over the third division and hypaesthesia over the third division of the right trigeminal nerve. There were no sensory disturbances on the left side of the face and no weakness of the masseter or pterygoid muscles on either side. On July, 12, 1938, the left spinal tract of the trigeminal

Strength of hair	Chin		Cheek		Forehead	
Touch	R.	L.	R.	L.	R.	L.
0.5 g./mm.	very occasional response on left face only					
1.0	0	4	2	4	1	6
2.0	0	9	0	8	3	4
3.0	0	7	0	8	3	6
4.0	0	8	0	8	8	9
5.0	0	10	0	9	7	10
Pain						
0.125 g.	0	0		0	0	0
0.5	0	4	0	4	1	2
0.75	0	7	0	8	0	2
1.0	0	9	0	7	2	2
2.0	0	9	0	10	3	4
4.0	0	10	0	9	5	9
10.0	0	10	0	10	10	10

nerve was sectioned under ether anaesthesia The patient had an uneventful recovery, but the pain in the first division was not relieved Sensory examination showed a marked hypalgesia over the third division less over the second and slight over the first division of the left trigeminal nerve To give relief from the pain, the supra orbital nerve was avulsed on July 16, 1938

Repeated neurological examinations showed that the abnormalities were confined to the sensory status of the face The findings using von Frey hairs before and after operation are as shown in the table at foot of page 244

Pre operative examination Patient is quite cooperative

Postoperative examinations July 15, 1938 (1 30 p m) Patient cooperative but responses only fairly consistent

Pain	L	L	L
0 75 g	1	1	5
1 0	3	2	4
2 0	1	3	9
4 0	1	(inattentive)	10
6 0	2	5	7

July 21 1938 (3 00 p m)   Cooperation good

Touch	L	L	L
1 0 g /mm	0	8	0
2 0	3	10	1
3 0	4	10	1
4 0	7	10	2
5 0	8	10	1
7 5	8	10	3
10 0	10	10	1
20 0			5
Pain			
0 5 g	0	0	0
0 75	1	1	1
1 0	1	1	1
2 0	0	1	1
4 0	0	3	0
8 0	2	0	0
10 0	1	2	0

Following operation the sensory disturbances seemed to be more severe than those in the first case as judged by the measurement by graduated stimuli Although the patient appeared to cooperate well his attention was readily distracted, and he fatigued easily For this reason the objective tests are not entirely reliable There was no doubt, however, in the minds of several observers, that there was a severe hypalgesia in the third division a lesser hypalgesia in the second division and before avulsion of the supraorbital nerve little sensory disturbance in the first division The appreciation of cotton wool was slightly but definitely diminished in the same areas

DISCUSSION

The sensory disturbances in the face after section of the spinal part of the trigeminal nerve are similar to those in the syndrome of the posterior cerebellar artery The findings suggest that the fibers in this tract carry im

pulses of pain and temperature and to a lesser extent touch. That touch is involved in these lesions is contrary to most of the earlier studies. Wallenberg[20] in summarizing the findings in one of his cases states that all sensory qualities were impaired but that pain and temperature were markedly reduced. Practically none of the other classical papers on the subject—Spiller[17] Gerard,[10] etc.,—suggest that touch is at all involved in these cases. This discrepancy may be explicable in one of at least three ways. The analgesia is so severe that the slight tactile disturbances may be overlooked. In most reported cases tactile sensibility was not examined by graduated stimuli, which are usually necessary to bring out the impairment. Individual variations in the tract and extent of the lesions may explain the failure of certain previous investigators using von Frey hairs to demonstrate the tactile impairment.

That, after apparently partial trigeminal tractotomy, the sensory disturbances in the face are much more pronounced in one part than in another favors the conception of Bergman,[2] van Gehuchten,[9] Davis and Haven,[6] and Sjöqvist[16] that there is a topical localization within the spinal tract of the fifth nerve. In both cases reported here the sensory disturbances were most marked over the lower part of the face, but in two of Sjöqvist's cases the analgesia was most pronounced in the first division. Because the exact site of the incision is unknown it is, of course, impossible to state from the present cases the precise topical arrangement of the spinal tract of the trigeminal nerve. The hypothesis of Bergman[2] and van Gehuchten[9] that the ophthalmic fibers lie in the ventral portion of the tract appears to be quite compatible with the present findings.

The arrangement of the sensory modalities within the spinal root of the trigeminal nerve is as yet a mystery. There is, however, ample evidence to suggest that, of the various categories of sensation carried in this tract, pain, heat, cold, pressure sense and touch are to varying degrees isolated. Several writers (Spiller,[17] Head and Holmes,[11] and Robinson,[14]) have reported cases of thrombosis of the posterior inferior cerebellar artery in which the appreciation of pinprick was lost, but heat and cold were undisturbed. Breuer and Marburg[3] have reported a similar case in which only the appreciation of cold was absent while pinprick and heat were appreciated. Sjöqvist[16] states that deep pressure sensibility was lost in some of his cases following trigeminal tractotomy, as it was in the first of the two cases reported here.

SUMMARY

1. The anatomical characteristics of the spinal tract of the trigeminal nerve are discussed. Its functional divisions are correlated with its anatomical structure.

2. The sensory disturbances over the face in two cases of thrombosis of the posterior inferior cerebellar artery are presented.

3. Trigeminal tractotomy is an operation which, under exceptional con-

ditions, is indicated for the relief of trigeminal pain The technique of the operation is discussed.

4. The sensory findings in two cases of trigeminal tractotomy are presented.

5 Analysis of the above cases and similar ones in the literature suggest that the spinal tract of the fifth nerve carries sensations of pain, heat, cold, deep pressure-pain and to a slight degree touch These sensibilities are probably isolated to some extent in the tract

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TIME CONSTANT OF EXCITATION AND VELOCITY IN SUPERNORMAL PHASE OF NERVE

H A BLAIR

*From the Department of Physiology, School of Medicine and Dentistry,
The University of Rochester, Rochester, New York*

THE SUPERNORMAL phase of the frog's motor nerve is often sufficiently long to permit measurement of a strength-duration curve for comparison with that of the same nerve at rest. This was first done by Adrian (1920) who concluded that the strength-duration curve of the supernormal condition differed from the normal only in that the voltage for each duration was lowered in a constant ratio. In other words, chronaxie was the same as usual during supernormality despite the lowering of the rheobase. The substantiation of Adrian's conclusion is important for at least two reasons: (i) in studying the relatively refractory period of nerve (including the supernormal phase) by means of shocks of short duration it is desirable to know what parameters of excitation should be used in expressing the divergence from normal (Blair, 1938, p. 136), and (ii) an easier method is provided for testing theories of propagation if the time constant of excitation does not vary appreciably as the velocity of the nerve impulse varies during the relatively refractory and supernormal phases. For these reasons, which will be discussed more fully later, voltage-capacity curves of the frog's sciatic nerve were measured both at rest and during the supernormal phase.

METHOD

The sciatic-nerve gastrocnemius muscle preparation was dissected and kept in the cold for at least one day before using It was then set up in Ringer's solution in an apparatus made from a block of paraffin with the muscle in one chamber and the nerve in a small trough connecting four chambers, in each of which a stimulating lead ending in a chlorided silver wire was inserted The upper pair of leads was connected to a stimulator consisting essentially of an 885 thyratron tube through which a 0 1 μF condenser in series with 13,000 Ω could be discharged by interrupting the negative grid bias The lower pair of leads was connected to a similar device with the same resistance but with a variable condenser enabling a voltage-capacity curve to be determined The grid of each tube was controlled by a commutator arranged in such a way that the interval between the stimulus to the first lead and to the second could be given any desired value In each circuit was a potentiometer in parallel with the nerve enabling the strength of the stimulus to be controlled The circuits were sufficiently independent at the nerve so that a sub-threshold shock over one pair of leads had no observable effect on the threshold stimulus over the other The measurements were made by determining first the normal threshold for a given capacity on the lower pair of leads, then the threshold at the end of a given interval after a maximal shock over the upper leads Then if a voltage capacity curve was required the procedure was repeated for other capacities, or if the supernormal phase was to be determined the procedure was repeated for other intervals In either case the muscular response was used as an index If the interval was short the threshold for minimal summation due to the second shock was determined or if the interval was long the threshold for a second response was observed The speed of the commutator was such that a pair of shocks could be delivered every 8 sec

The Ringer's solution contained 0 65 g Na Cl, 0 01 g KCl, 0 015 g Ca Cl$_2$, 0 192 g Na$_2$ HPO$_4$ (12 H$_2$O) and 0 0184 g Na H$_2$PO$_4$ (H$_2$O) per 100 cc

THE SUPERNORMAL PHASE

Figure 1 illustrates the supernormal phase of a nerve which has been isolated for at least several hours. It is considerably exaggerated over those taken soon after dissection, both in duration, which may be a large fraction of a second, and in its maximal value, which is commonly about 35 per cent in terms of the strengths of the test shocks, or 100/65 (as it is usually expressed) in terms of excitability. Figure 1 shows that the disappearance of the supernormal state is sufficiently slow at most parts of the curve so that no great change occurs in 0.01 sec. which is as long as the utilization time of the rheobase for this tissue will ordinarily be. Therefore, the strength-duration curve taken at most phases of the supernormal state will not be markedly distorted by progressive changes of the state even with the longest stimuli.
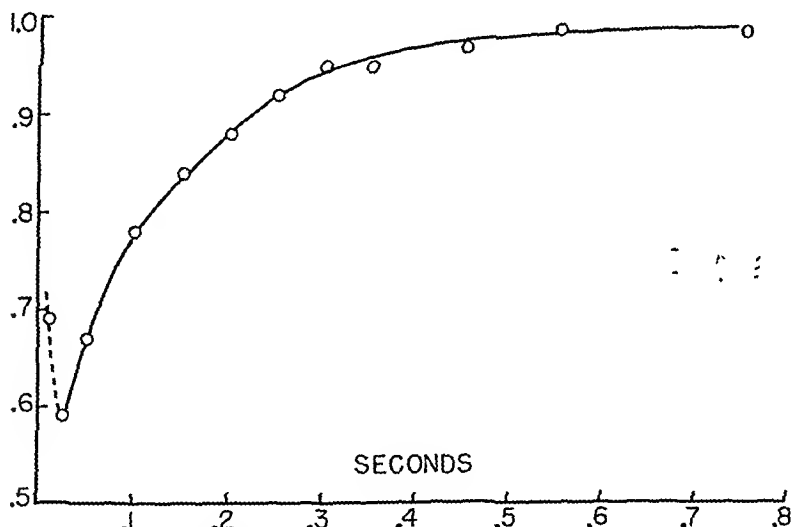
FIG. 1. Unit value of the ordinates is the normal threshold with a stimulus of time constant 0.3 msec. The ordinates of the curve are the threshold strengths of similar shocks during the supernormal phase following a maximal shock at zero time. The early part of the refractory period was not determined.

The voltage-capacity curves

In the voltage-capacity determinations, the longest stimulus used had a time constant of 13 msec. since the resistance was always 13,000 Ω and 1μF was the largest capacity. The voltage required with this capacity was close to the rheobase as is evidenced by the fact that usually the voltage had to be raised only slightly with 0.5μF. Figure 2 gives a typical comparison between voltage-capacity curves taken at rest and during the supernormal phase at 75 msec. after its beginning. Superficially there is no well defined change in chronaxie, but when the ratio of the voltages for each capacity is

determined it is found that it increases definitely as the capacities become smaller. This is illustrated in the same figure for the same data and for a number of cases in Fig. 3.
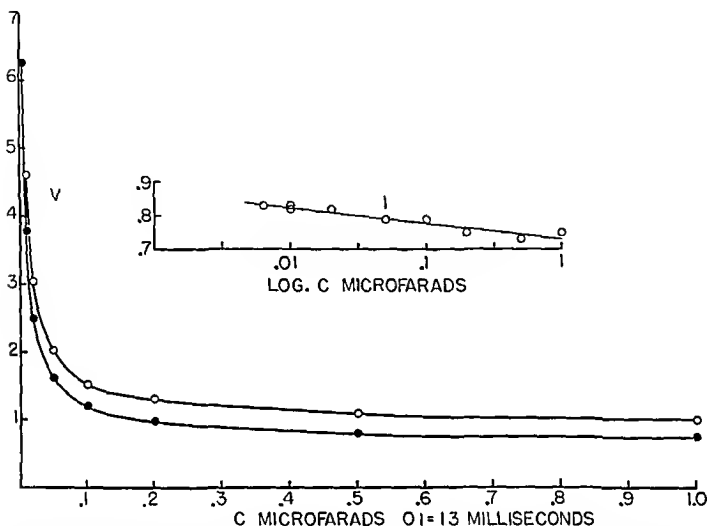
FIG 2 The normal voltage-capacity curve (in open circles) on a scale with the rheobase equal to unity and one obtained 75 msec. after the beginning of the refractory period (in dots). The ratio of the supernormal voltage to the normal for each capacity is plotted above against the logarithm of the capacity. Chronaxie is marked by a vertical bar The ratio increases from 0 74 to 0 79 on going from the longest stimuli back to chronaxie

In Fig. 3, the abscissae are capacities on a logarithmic scale for convenience and the ordinates are the ratios of the voltage thresholds during the supernormal phase to those of the normal nerve at the same capacity. Each curve represents data for a different interval between the initial and test stimuli corresponding in general to a different stage of recovery. Each one is on a different nerve, however, so no relation such as that shown in Fig. 1 can be obtained from these cases. The ratio of the thresholds usually increases considerably in going from the largest to the smallest capacity and by several per cent in going from the rheobasic durations to chronaxie, which is marked by a vertical bar in each case. To answer the question how much change in the time constant of excitation is involved it is convenient to use the equation of the voltage-capacity curve.

$$\frac{V}{R} = (crk)^{1/crk-1} \tag{1}$$
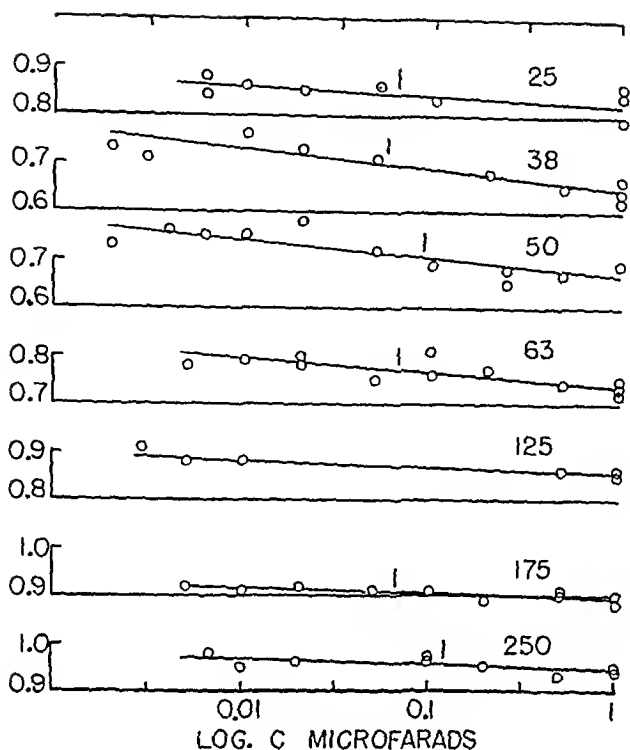
FIG. 3. The ratio of the threshold voltage during the super-normal phase to the normal against the logarithms of the capacities. The resistance of the circuit was always 13,000 Ω so $0.1\mu F$ equals 1.3 msec. The number above each curve is the time in msec. from the beginning of the refractory period. The vertical bars mark the chronaxies.

V, being the initial voltage of the condenser stimulus, R, the rheobase, and cr, the time constant of the circuit, derived from the assumptions (1) that the excitatory state E grows in response to the stimulus according to

$$\frac{dE}{dt} = KV - kE \tag{2}$$

K and k being constants, and (2) that E becomes adequate on attaining the value h (Blair, 1932). By substituting in equation (1), it will be seen that $V = 2R$ when $crk = 2$. In other words, $k = 2/\text{condenser chronaxie}$. On differentiating 1 with respect to k and putting in the condition $V = 2R$ and $crk = 2$.

$$\frac{dV}{dk} = \frac{1}{k}(2 - 4 \log_e 2) = \frac{-0.8}{k} = -0.4\,cr$$

which is the variation of V with k in the region $V = 2R$. The value of h at

20°C. is about 3000 sec.$^{-1}$ since the chronaxie, $cr = 0.7$ msec. approximately. Therefore $dV/dk = -1/3700$ approximately. Thus in fair approximation, if V, when equal to 2, changes by 0.02 or 1 per cent, k changes by 0.02×3700 $= 74$ or 2 per cent.

In Fig. 2, V during the supernormal phase is about 5 parts in 75 or 7 per cent larger at the chronaxie region than it would be if the excitability had not changed. Therefore, according to the argument above, k is reduced
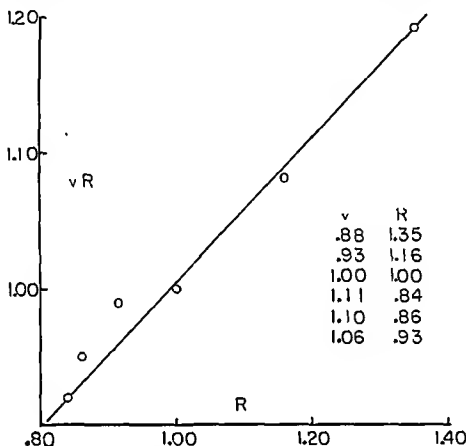
FIG. 4. The velocity v times the shock strength R plotted against R for the data inset. The normal velocity and the normal shock strength are each given the arbitrary value unity. These data of the velocity during the recovery period in frog's nerve are from a graph given by H. T. Graham, (1934, p. 234). The upper points are during the subnormal and the lower during the supernormal phase.

by about 14 per cent in this particular case, or chronaxie is similarly increased. It will be seen in Fig. 3 that k is always smaller during the supernormal phase although not by more than about 5 per cent when the recovery is sufficient to make the rheobase ratio greater than 0.85. This is the range in which most previous work has been done and Adrian's conclusion is not seriously invalid here. It will be seen, however, that the assumption of an invariable excitability is far from valid when the supernormality is large and that in consequence the form of the recovery curve depends on what type of stimulus is used to determine it, for if cr becomes so small that crk is $\ll 1$ in equation (1), $V/R = 1/crk$, but $R = kh/K$ so $crV = h/K$. There-

fore, short stimuli of constant durations measure the recovery in terms of h/K essentially, while long shocks such as the rheobase measure kh/K including changes in the excitability k as well. Most measurements of the recovery cycle have been made with short shocks enabling the phenomenon to be described in terms of h/K only. A complete description of the cycle must include also, however, the variation of k as well.

It will be observed that since $crV = h/K$ when cr is small, and $R = kh/K$, $V/R = cr/k$. Thus using subscripts 1 and 2 for the normal and supernormal conditions respectively, and assuming the same value cr for a brief stimulus in each, it will be observed that

$$\frac{V_2}{V_1} = \frac{k_1}{k_2} \times \frac{R_2}{R_1}$$

and taking for the data of Fig. 2, $V_2/V_1 = 0.83$ at $cr = 0.01$ and $R_2/R_1 = 0.73$ $k_1/k_2 = 1.14$, a result similar to that obtained above by an alternative method.

THE VELOCITY PROBLEM

Rashevsky (1931, 1933), using the hypothesis that the nerve excites itself electrically to effect propagation has developed a general method for expressing the velocity of the nerve impulse in terms of the parameters of excitation and the physical properties of the nerve fibre. Using the excitation law given by equation (2) this leads to the expression for the velocity v,

$$v = \frac{I - R}{R} \times \frac{k}{\alpha} \tag{3}$$

in which I is the action current, R the action current rheobase, α includes various physical properties and k is the excitability. There are difficulties in testing this expression by means of data from different fibres in a trunk because it is not known how the physical properties vary from fibre to fibre. During the recovery cycle, however, one can study the variations of the velocity in a given fibre whose physical properties and whose action current probably do not change appreciably, while the quantities R and k do. In particular, if k changes relatively little and I and α are assumed constant, there is the linear relation

$$vR = \text{constant } (I - R) \tag{4}$$

connecting the velocity and the rheobase or, which is the same thing under these conditions, the shock strength.

A set of data by Graham (1934) are plotted according to equation (4) in Fig. 4. The agreement is not bad, but since the supernormal phase is 16 per cent at the maximum, k is probably reduced by 5 per cent or more at that stage so it cannot be considered a constant. It can be seen from the

previous discussion that in putting $kh/K = R$ in the denominator of equation (3), equation (4) may be written $vS = $ constant $\times (I - R)$, S being the strength of a brief shock Thus the way to correct for the effect of k being reduced in this case will be to move the lowest two points a few per cent toward the left, leaving the ordinates the same, which will not make the approximate linearity any worse

This single result cannot be taken as substantiating Rashevsky's theory to any great extent It is given more as an illustration of the method of testing the electrical propagation hypothesis in this way which, is relatively simple, even though the excitability k is a variable during recovery, for it is necessary, according to the equation just above, to measure only S and R at any stage of recovery in order to take the variations of k into account There is a possible difficulty which is perhaps impossible to solve directly, namely as to whether k varies during the early, subnormal, phase of recovery For this reason it may be necessary to confine the application of equation (4) to those parts of recovery during which the variations of k are directly measurable The supernormal phase can be made great enough, however, to give a range of velocity variations adequate for the purpose

SUMMARY AND CONCLUSIONS

Comparison of normal voltage capacity curves from the frog's sciatic nerve with those taken during the supernormal phase of recovery shows that the time constant of excitation is decreased (chronaxie is increased) during the supernormal phase by as much as 15 per cent when the supernormality is more than 30 per cent Therefore a complete description of the recovery in nerve must take variations of the excitability into account as well as the other excitation parameters Also the supernormal phase will be greater in depth, as determined by stimuli of long than of short duration, since the latter tend to ignore changes in the time constant A method is described for testing the validity of Rashevsky's electrical self-excitation theory of nerve impulse propagation by relating the altered excitation parameters to the altered velocity during the recovery cycle A single set of data by Graham lends support to the hypothesis

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CEREBRAL BLOOD FLOW DURING INDUCED EPILEPTIFORM SEIZURES IN ANIMALS AND MAN*

WILDER PENFIELD, KÁLMÁN von SÁNTHA and ANDRÉ CIPRIANI

Department of Neurology and Neurosurgery, McGill University and the Montreal Neurological Institute, Montreal

THE MECHANISM of an epileptic seizure presents a complicated physiological problem that should be studied in the epileptic patient and supplemented by analysis of experimental seizures in animals. The modification by Gibbs[1] of the thermoelectric method of measuring blood flow has provided a technique of study of the cerebral circulation during seizures in animals which has been adapted with few changes to an analogous study of epileptics during craniotomy. Preparatory to our use of the method von Sántha and Cipriani[11] used it with certain minor modifications to analyze simple motor responses in animals. They found that increased function (nonconvulsive) produced by cortical stimulation, was associated with temporary increase of local blood flow. Recently Gibbs[1] and Gibbs, Lennox and Gibbs[2] have used the technique to analyze blood flow in the parietal cortex of animals and the rate of flow through the jugular vein of man during epileptiform seizures. They found no evidence of decreased blood flow preceding an attack thus excluding a generalized cerebral ischemia at this time. They concluded also that during a seizure there was a greater total cerebral blood flow.

Approaching the problem from a different point of view and using different material our results nevertheless conform in a general way to theirs. We can also add further details in regard to delimitation of convulsive discharge and interpretation of the discharge mechanism.

EXPERIMENTAL STUDIES

In our experiments seizures were induced by electrical stimulation instead of by convulsant drugs. Thus we knew the focus of initiation of each convulsion and there was no question of the direct effect of a drug on the vascular system. A response was considered to be convulsive when it continued or spread after the cessation of cortical stimulation. The blood flow in different areas of the brain was measured with one or two of Gibbs' heated thermocouples with certain precautions to be mentioned below.

TECHNIQUE

Cats (35 in number) and monkeys (15 in number) were used, as a rule under Dial anesthesia although several of the monkeys were operated upon under local nupercaine

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anesthesia. The dose of Dial given intraperitoneally was 0.4–0.5 cc. of a 10 per cent solution per kg. for cats, and 0.4 cc. for monkeys. In several cases the anesthesia was made lighter in order to increase the excitability of the cortex for electrical stimulation. This was done by the administration of intravenous metrazol (0.2–0.5 cc. of a 10 per cent solution). In most of the experiments a unilateral left craniotomy was performed, in the remainder the skull was opened bilaterally. The heated couple was inserted into the exposed cortex or in some deep structure of the brain. In the case of a deep structure its position was later verified by autopsy. In the early experiments the fixed temperature junction was placed in an indifferent part of the brain. Later on it was immersed in a thermos bottle filled with melting ice. The alterations in the temperature of the tip of the couple produced by changes in blood flow were recorded on a kymograph drum together with blood pressure and respiration.

Owing to the sensitivity of the recording device (0.0025°C. per mm. deflection) a series of experiments was conducted in order to account for and to eliminate artefact with the following results: When the heated couple was placed in grey matter 1 or 2 mm. below the brain surface it was necessary to shield the operative field from draughts. The shield used was partly made of glass so that the cortex could be observed, and a bipolar electrode was incorporated in the shield in such a manner that the cortex could be stimulated over its exposed surface without the removal of the shield. In these preliminary control experiments the stimulating current* heated the tissue around the electrode to an appreciable extent: thus stimulation close to the couple could simulate a decrease in blood flow. With this in view a minimum stimulating voltage was always used without stimulating too close to the couple. We further observed that the circulating blood was at a higher temperature than the superficial layers of the exposed cortex. Thus the heated couple when inserted superficially could indicate either an increased or decreased blood flow depending on whether it was at a higher or a lower temperature than the circulating blood. The proper temperature for the tip of the thermocouple should be checked in each case by the preliminary use of adrenalin and histamine intravenously; the former could always be relied upon to increase the cerebral circulation and the latter to decrease it under Dial anesthesia.

Results

Experiments type 1 (Monkeys). The thermocouple was placed within the grey matter of the middle third of the precentral gyrus corresponding to the inferior part of the hand area. When contralateral leg movements were produced by stimulation of the leg area the galvanometer showed no change in blood flow. When the cortex just above the sulcus precentralis inferior was stimulated, eliciting hand and arm movements, a definite increase in circulation was recorded. A more marked increase in circulation resulted from stimulation of the cortex lying above the sulcus precentralis superior when there was a 35 second after-discharge involving the contralateral hand, arm and trunk. It should be pointed out that the three stimulation points mentioned above were practically equidistant from the thermocouple. Thus the phenomenon cannot be attributed to proximity of the stimulating electrode.

Experiments type 2 (Monkeys). A bilateral craniotomy was performed. The couple was placed in the left motor cortex. An increase in blood flow was recorded when unilateral right-sided convulsions resulted from stimulation of the left motor or premotor cortex (IL in Fig. 1). On the other hand there was no significant change when a seizure was produced on the left by right motor and premotor stimulation, but the blood flow record alterations were simply parallel to those of the systemic blood pressure (CL Fig. 1).

On increasing the intensity of the stimulus, or on raising the excitability of the cortex with metrazol generalized convulsions followed unilateral cortical stimulation (Fig. 1), in which case a blood flow increase was recorded in the left motor cortex irrespective of the side from which stimulation had precipitated the fit (Fig. 2). There was no difference in the magnitude of the circulatory change but there was a delay of a few seconds in the change resulting from right-sided stimulation. The results were the same whether the fit had been produced by stimulation of the motor, premotor or postcentral cortex.

Experiments type 3. The thermocouple was placed in the premotor cortex, the postcentral cortex and outside of the sensorimotor cortex. Generalized convulsions were pro-

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* The stimulating current was from a thyratron, "a universal precision stimulator" described by Schmitt and Schmitt (*Science*, 1932, *76*: 328).

duced. In monkeys the premotor and postcentral cortex showed circulatory changes similar to those found in the precentral cortex, with no delay on ipsilateral stimulation. The area rostral to the premotor cortex, *i.e.*, areas 8, 9c and 9d, showed smaller changes with a definite delay. This difference was even more marked in area 10a. The orbital cortex revealed no definite increase in blood flow. No circulatory change was recorded in area 7 of cats. In area 7 of monkeys (gyrus supramarginalis) there was increased circulation when a generalized fit was produced by stimulation of the ipsilateral postcentral gyrus. Only a small change occurred when a similar fit was produced from the contralateral side.

We must conclude that the change in the supramarginal gyrus resulting from ipsilateral stimulation resulted from some intracortical connection and was not related to the
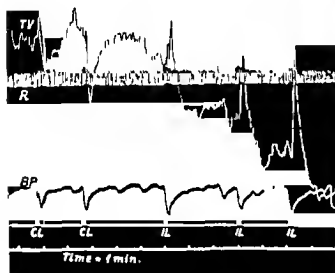
FIG. 1. (Expt. 1590.) Changes in blood flow of the left motor cortex (monkey) associated with unilateral seizures produced by stimulation of contralateral (CL) and ipsilateral (IL) precentral gyrus. TV = temperature variations of couple, a fall in the curve indicating decreased flow and a rise the reverse. BP = systemic blood pressure taken from femoral artery. R = respiration.
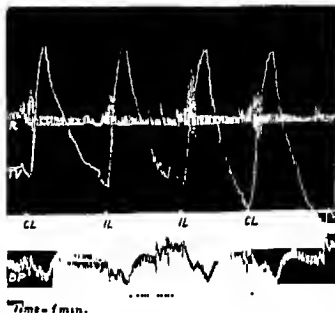
FIG. 2. (Expt. 1590.) Blood flow changes in the left motor cortex (monkey) after contralateral (CL) and ipsilateral (IL) stimulation of the frontal cortex lying just anterior to the precentral gyrus, resulting each time in a generalized convulsion. These results were obtained after the animal was sensitized with metrazol. Sensitivity of blood flow recorder one-third of that in Fig. 1. TV = temperature variations of couple resulting from blood flow change; rise of curve indicates increased blood flow. BP = blood pressure taken from the femoral artery. R = respiration.

seizure. In a few cases the effect of stimulation of areas lying outside the sensorimotor field on the circulation of the motor cortex was studied. No circulatory change was observed in the motor cortex when areas 10a, 9a, 5 and 7 were stimulated.

Experiments type 4. The thermocouple was placed in some of the subcortical ganglia, the caudate nucleus and the thalamus of cats, the thalamus, putamen and pallidum of monkeys. During generalized convulsions these subcortical centers showed a marked increase in circulation, greater even than that observed in the cortex. In cats following contralateral stimulation, which produced a unilateral motor response, there was no circulatory change in the caudate and thalamus. A similar ipsilateral stimulation produced a marked increase in circulation. In one monkey we were able to conduct such an experiment in the pallidum with similar results.

When there was a generalized convulsion the change in blood flow in the above ganglia was the same whether the left or right motor cortex initiated the seizure. We also observed

and have described elsewhere[11] that only certain parts of the thalamus showed a circulatory change in response to motor stimulation on the ipsilateral side. In the pulvinar and nucleus lateralis posterior no significant change could be observed.

Experiments type 5 (Monkeys). In a few cases we were able to record blood flow in monkeys during spontaneous fits. The animals were given metrazol intravenously which was followed by a severe convulsion. After this generalized convulsions could be produced by weak electrical stimulation. These electrical fits lasted from a half to one minute. Subsequently the animals continued to have seizures at about 5 minute intervals without stimulation. We recorded changes of blood flow in the motor cortex, the putamen and the medial nucleus of the thalamus. There were no pre-convulsion phenomena and the blood flow record differed in no way from that elicited by stimulation of the motor cortex. The subcortical ganglia again showed a greater reaction than the cortex.

In interpreting the records obtained in the brain we have paid special attention to the nature of the tissue surrounding the thermocouple. If the couple is entirely surrounded by white matter the changes are small and sluggish and tend to be influenced by the systemic blood pressure in a passive manner. In the subcortical centers the proportion of grey to white matter varies. In the cortex itself a thermocouple too deeply placed may be partially embedded in the underlying white matter, resulting in an apparently reduced reaction.

Discussion of experimental evidence

The alteration in blood flow measured by the thermo-electric method during convulsive seizures depends on the function of the surrounding tissue. Some areas of grey matter seem to play little or no rôle during a seizure, even a so-called generalized seizure. The circulation change depends further upon the structure of tissue and the capillary bed which surround the couple. According to Craigie[5], Cobb and Talbott[6] and Lindgren[7] the vascular supply of the grey and the white matter is altogether different. The grey matter shows considerable inequality in supply but the most poorly vascularized grey matter contains 50 per cent more capillaries than the best vascularized white matter. In general the sensory and intercalary centers are better vascularized than the motor centers. The cortex, particularly its fourth layer, is well vascularized. According to Spatz[8] the blood supply of the striatum is probably still more abundant. Dunning and Wolff[9] believe that the blood supply of a given center is directly proportional to the number of synapses. In the cerebral cortex the thermocouple is placed in a grey layer that is thin and superficial. In the thalamus the couple is deep and more completely surrounded. That these factors are relatively unimportant however, is shown by the injection of intravenous adrenalin which induces a similar increase of flow both in the cortical and subcortical centers. This is shown by the simultaneous tracings in Fig. 3.

Generalized electrical seizures and certain spontaneous seizures in animals are accompanied by increased circulation in certain parts of the brain. Reference to type 3 experiments above shows that outside of the sensorimotor cortex other areas of cortex may show little or no circulatory change when a fit is produced by stimulation in the sensorimotor area, and conversely stimulation in these outside areas fails to produce a seizure. Gibbs, Lennox and Gibbs[2] suggested that this blood flow change might be passive, due either to increased blood pressure, or to general increase of CO_2. According to our observations neither of these explanations would seem ade-

quate since we are obviously dealing with localized circulatory changes due to some focal cause. The only plausible explanation of this increased circulation, which begins from about 4–10 sec. after the first convulsive movement, and continues after the attack has ceased, seems to be the increased neuronal activity of the region in question. This augmented function must be associated with increased metabolism which in turn produces the chemical stimulus for the vasodilatation. The circulatory increase produced by a seizure is greater in the involved subcortical centers than in the involved cortex. The sluggish and small responses of the white matter are to be explained by poor vascularity and by low rate of metabolism.

OBSERVATIONS ON HUMAN BEINGS

A study of circulation during fits in man has been carried out over a period of two and one-half years whenever a suitable case presented the opportunity. The tiny thermocouple was embedded within the brain substance at operation. But inasmuch as the observations were always secondary to more urgent clinical problems only a few of the records are above criticism and therefore suited to the present study. At the beginning we were assisted by Dr. Norcross whose experimental work on blood flow has already been published.[3]

The operative procedure employed for cortical exploration of selected cases of focal epilepsy and the therapeutic results have been outlined elsewhere by Penfield.[10] A large area of one hemisphere was exposed under nupercaine analgesia. Electrical exploration was then carried out with a bipolar or unipolar electrode using the thyratron stimulator employed in the experimental study described above. The kymograph drum galvanometer and recording apparatus were set up in an adjoining room and the time of stimulation, of onset of seizure, etc. were signalled directly to the drum by an observer in the operating theater.

Time = 1 min.

FIG. 3. Blood flow increase in the putamen and cortex (monkey) after intravenous adrenalin (0.05 mgm). P = blood flow change in the putamen. C = blood flow change in the cortex. R = respiration. BP = blood pressure taken from the femoral artery.

CASE 1. F.H. A young man of 25 years received a head injury 2 years before operation. Focal seizures appeared 6 months later. A large right osteoplastic craniotomy was carried out and a meningocerebral cicatrix was exposed involving the lower one-third of the Rolandic cortex. The thermocouple was inserted into the grey matter at point 1 Fig. 4. At E stimulation produced trembling in large finger and thumb of the contralateral hand;

at G there was "electrical" feeling in hand and wrist. Stimulation at O caused the patient to say suddenly "I got the feeling I get in my attacks, *a twisting round feeling of the eyes.*" The one eye which could be seen by the observer closed, squinted and then looked upward.

Attack I. Stimulation repeated at O (Fig. 4) produced a slight attack. The patient cried "Oh" and for 45 sec. there was a good deal of emotion and he could not speak clearly. At 15 sec. he whispered "It felt like an attack. It felt as though the head were turning of its own accord."

Attack II. Stimulation at X continuing 3 sec. produced similar attack but a little
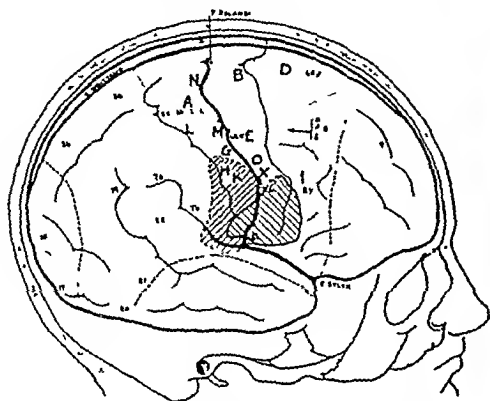
Fig. 4. (Case 1.) Thermocouple positions are represented by points 1 and 2. Letters refer to stimulation points. The shaded area is the approximate location of the epileptogenic lesion.
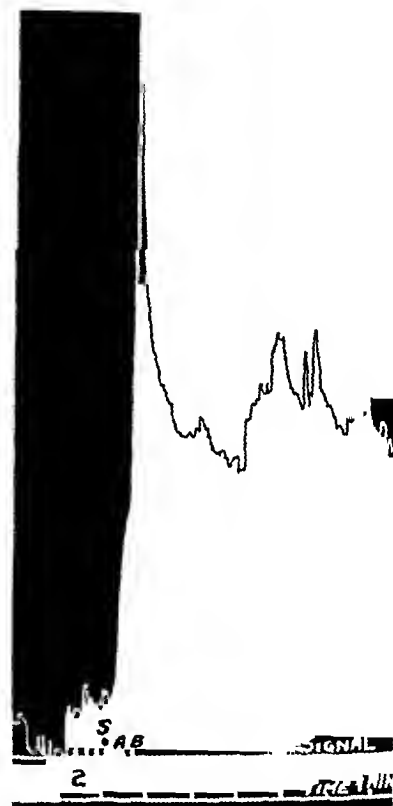
Fig. 5. (Case 1.) Attack III. Blood flow record with the thermocouple in position 2. Stimulation was at point X (Fig. 4) indicated by S on the signal line. The attack was recognized by the operator at A on signal line and was over at B.

more severe. The thermocouple was now removed from point 1 and inserted into the cortex at a point 3 mm. below X, that is at point 2. The wire carrying the thermocouple was passed through the pia and obliquely inward 1.25 mm. so that the couple remained superficial. The act of inserting the couple which was now quite near the scar was sufficient to produce a moderately severe focal attack of 17 sec. duration similar to the others. No blood flow record was made of this.

Attack III. Stimulation at X produced a more severe attack but focal like the others. The patient said at the close "Same kind of attack," but his head and eye were turned forcibly to the left side and there were clonic movements of the left arm. Just before stimulation visible pulsation in the superficial arteries of the brain was quite active. All visible pulsation disappeared with the onset of the seizure and returned 11 sec. later. Pulsation on its return was violent as is usually the case.

The blood flow as measured at point 1 showed a small increase coincidental with Attack I. The increased flow was a little greater with Attack II. When the thermocouple was reinserted at point 2 the sensitivity of the flow record was purposely decreased but Attack III was associated with a marked increase in blood flow (Fig. 5) which reached its peak at 18 sec., during the time when visible arterial pulsation had disappeared. These were focal seizures. The warmed thermocouple was in the discharging area of cortex and it indicated more rapid cooling which is taken to mean greater flow of blood through the tissue during the seizure.

FIG 6 (Case 2) Photograph of the field at operation The thermocouple with attached black wires can be seen inserted just above S and passing under ticket (X) The tickets were laid on the cortex to mark the sites from which electrical stimulation produced sensory or motor response

CASE 2 M M A young man suffering from habitual epileptic seizures had a small traumatic lesion in the supramarginal gyrus After right osteoplastic craniotomy the thermocouple was inserted to a depth of 1 mm at the site of superficial injury (Fig 6) Stimulation at X in the postcentral gyrus produced a focal attack characterized by loss of consciousness, turning of eyes to left and a little pulling of the mouth to the left This lasted 7 sec when he spoke again but it was followed almost at once by two repetitions of the attack each with conjugate deviation of the eyes There was an associated increase of blood flow which was of somewhat longer duration than in the previous case No change in the pulsation of the superficial arteries was observed

Figure 7 shows the blood flow record At A on the signal line the cortex was stimulated at point A as shown by the ticket on the brain in Fig 6 The result was sudden marked closure of the hand and flexion of the elbow Stimulation was next carried out at X on the brain which corresponds with S on the signal line This produced the seizure but the operator was only certain that it was a seizure about 40 sec after stimulation when he signalled to the drum at point X on the signal line At B and again at C the operator recognized that a second and a third small spontaneous fit were occurring and it is seen that there was a further increase of blood flow corresponding with each recurrence

CASE 3 M O An example of blood flow record taken outside of the discharging area may be given Here the thermocouple was placed in area 19 of the
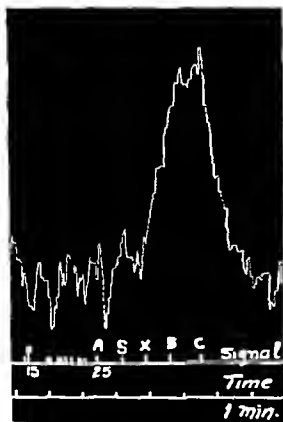
FIG 7 (Case 2) Blood flow record during an attack resulting from stimulation at point (X) in Fig 6 On the signal line the instant of stimulation is indicated by S, the time of recognition of fit at X

parietal lobe 10 cm. from the stimulation point which was in area 6aβ in the frontal lobe. The attack was focal in nature consisting in contralateral movements of arm and face. There was no evidence of alteration in blood flow in the tissue about the thermocouple although the apparatus was recording satisfactorily as shown by the cooling effect each time the cortex was sprayed to keep it moist. This would suggest that the blood flow alteration is focal in a focal discharge.
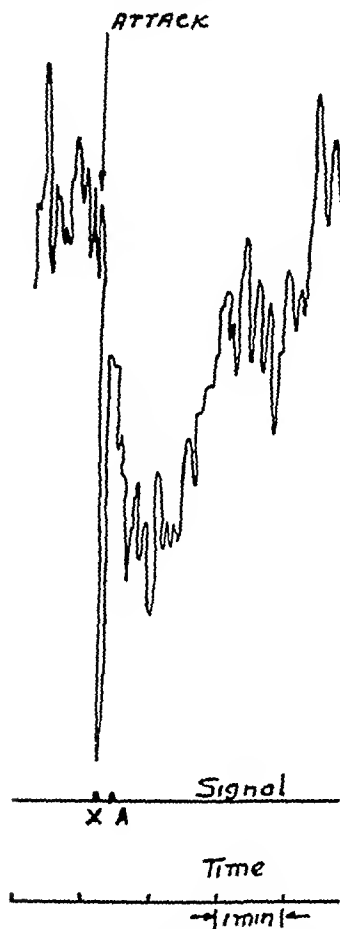
FIG. 8. (Case 4.) Blood flow record during an attack. X denotes instant of stimulation and A the end of the attack. For explanation of decrease in blood flow see text.
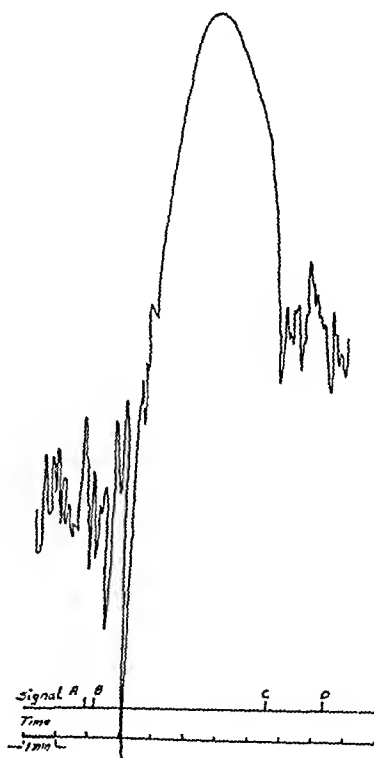
FIG. 9. (Case 5.) Blood flow record during an attack. Stimulation took place at A on the signal line. At B the attack was first recognized by the operator.

CASE 4. L.B. A young woman was found to have a deeply situated infiltrating tumor beneath the left postcentral gyrus. Stimulation of this gyrus however gave sensory responses with a lower threshold than the precentral. The thermocouple was placed in the frontal lobe 6 cm. anterior to the central fissure. A small focal attack produced by stimulation in the postcentral gyrus at a distance of 6 cm. was associated with no alteration in blood flow about the couple. A second attack however was produced by stimulation at a point 2 cm. posterior to the couple, the stimulation being continued for 7 sec. The attack lasted 8 sec. after its recognition which followed withdrawal of the stimulator and con-

sisted in clonic movements of the right upper extremity which the patient said resembled her habitual attacks.

The blood flow about the thermocouple showed a decrease (Fig. 8) rather than an increase. It may well be that the discharging state of the cortex which took origin at the point of stimulation spread only backward toward the central fissure and so never involved the tissue surrounding the thermocouple. If that surmise is correct then the temporary decrease in blood flow might indicate a contrary alteration of blood flow peripheral to the discharging area.

CASE 5. J.R. A young boy of 12 was subject to epileptic seizures beginning in the right hemisphere. The focus proved to be an area of circumscribed softening situated low down at the junction of the right temporal and occipital lobes. The thermocouple was placed in the lower postcentral convolution at a distance of about 12 cm. Only one attack was produced and that followed stimulation in the softened area mentioned above. The onset of the attack resembled that of his habitual major seizures. He swallowed, said he felt nauseated, vomited and his pupils were noted to be dilated. The pupils then contracted suddenly to a very small size, the eyes deviated slowly to the left, the face pulled to that side and he was apparently unconscious.

The thermocouple lying in the Rolandic cortex, which corresponds roughly with face representation, was a considerable distance from the stimulated focus. Convulsive movement of the face to the opposite side was the final phenomenon of the fit. The blood flow showed little alteration for almost a minute, then there was a sharp drop followed by a long continued increase of flow lasting over 4 min. (Fig. 9). The discharging state probably moved across the cortex toward the Rolandic region, as it usually does, so that the thermocouple was first on the outskirts and later within the area of epileptic discharge as the disturbance travelled forward. Here again there seems to have been slowing of circulation at the periphery.

GENERAL DISCUSSION

In chronic epileptics focal fits may be induced by electrical stimulation of the cortex which are outwardly identical with the habitual fits of the patient in question. During the seizure there is an increase in circulation within the circumscribed area of cortex which is involved in the "discharge" that produces the fit. This increase begins within the first minute after the precipitating stimulus with no preliminary decrease in flow and it outlasts the clinical evidence of the seizure. More distant areas of cortex may show no alteration in blood flow as in Case 3 above, or there may be a decrease in circulation of short duration at the periphery of the involved area of cortex as in Cases 4 and 5. Whether this peripheral decrease is an invariable phenomenon it is impossible to say from our limited observations. No initial decrease occurs at the focus where the attack begins.

In animals epileptiform seizures were accompanied by a constant circulatory increase in the motor cortex (using the term motor broadly). A similar but more marked increase has been observed in the caudatum, putamen, pallidum and thalamus. This blood flow increase appeared to be limited to the contralateral hemisphere in cases of unilateral convulsion. In generalized convulsion the change was equally present in both hemispheres. This increase in circulation started a few seconds after the first muscular movements and stopped soon after the attack was over. No significant circulatory change could be found in the white matter. The similarity in reaction between the unanesthetized human cortex and that of animals under Dial indicates that the Dial did not essentially alter the reactions in the

animal experiments and it further suggests that conclusions drawn from the experimental evidence may be applied to clinical epileptic seizures.

In the case of the experimental seizures we have concluded above that the change in circulation is secondary to the sudden increase in neuronal discharge taking place during the convulsion, and that this increase of neuronal activity was even greater in subcortical centers than it was in the cortex. This is in conformity with the findings of von Sántha and Cipriani[11] who showed that cortical stimulation which produces a simple motor response, without convulsive after-discharge, also produces a demonstrable increase in blood flow both in the cortex and in the related subcortical centers. The evidence of increased function both in the cortex and in the subcortical centers is contrary to the theory that would explain an epileptic convulsion as a release of subcortical centers from cortical control.

That there may occur in both the cerebrum and cerebellum of epileptic patients at some time vascular spasms is suggested by the well-known work of Spielmeyer and his pupils. Also it was pointed out by Penfield[14] that arterial constrictions are sometimes seen in the exposed epileptic cortex but that they appear after a convulsion and not during it. During an epileptic seizure in man the most frequently observed objective change in the brain is cessation of visible pulsation of pial arteries.[15] Whatever the explanation of this phenomenon may be, it is not due to decreased blood flow as shown in Attack III of Case 1 above. The evidence of our present communication indicates that vasospasm plays no rôle during a seizure nor is there evidence of widespread anemia following it. Vascular spasms and anemias may be concerned in the pathological background of epilepsy but at all events they play no rôle in the actual mechanism during a seizure.

Even though increased circulation does occur there is no reason to conclude that the enormously increased need for oxygen on the part of the neurones is completely satisfied (Penfield[15]). The tissues may still be in a state of severe anoxaemia. For example, a muscle suddenly called into use may double its circulation but if the muscle needs three times its resting circulation the muscle tissue will be in a state of functional anoxaemia.

SUMMARY

Increased blood flow of the area of brain involved seems to be the invariable accompaniment of experimental convulsions in animals and of epileptic seizures in man. This change in blood flow does not apply to the whole brain and is therefore not a phenomenon secondary to changes in general circulation or respiration. The circulatory increase begins a little time after the onset of local neuronal discharge and may be interpreted as the direct result of increased ganglionic activity.

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ROLE OF THE SYMPATHETIC SYSTEM IN REFLEX DILATATION OF PUPIL*

BORIS URY AND ERNST GELLHORN

Departments of Physiology and Psychiatry, College of Medicine, University of Illinois, Chicago

IN A preceding paper (Ury and Gellhorn) it was shown, in the rabbit, that the reflex pupillary dilatation produced by weak stimuli was solely due to an inhibition of the parasympathetic tone and not to an excitation of the sympathetic. The problem presented itself how far these results are applicable to other species under various conditions. The vast literature on this subject contains a wide disagreement concerning the rôle of the sympathetic and parasympathetic mechanisms in the pupillary dilatation in man and animals when pain stimuli are applied. Bechterew (1883), Braunstein (1894) and more recently Bain, Irving and McSwiney (1935) emphasize the importance of the inhibition of the parasympathetic, whereas Anderson (1904), Luchsinger (1880) and Dechaume (1937) claim that the sympathetic is responsible for the pupillary reaction since it is absent after the cervical sympathetic has been cut. Gellhorn and Darrow (1939) observed in cats that faradic stimuli applied to afferent nerves cause a dilatation of the normal and of the sympathectomized pupil, the former dilating somewhat more than the latter. Therefore, in addition to the inhibition of the parasympathetic which is fully established, the intact sympathetic either maintains a tonic innervation or is reflexly excited. The experiments reported here attempt to decide this question.

METHODS

The experiments were carried out on 9 cats placed in wooden boxes of special construction to allow a rigid fixation of the head. Faradic stimuli (Harvard inductorium with 3 v. in the primary circuit) were applied either with a different electrode on the paw and an indifferent one on a shaved area of the back, or with both on the exposed sciatic nerve. The lids were held wide open by a retractor and the pupils photographed with a 35 mm. movie camera. Two signal magnets recorded respectively the period of stimulation and the time when the individual pictures of the eyes were taken. The eyes were magnified 2.3 times but graphs in this paper are reduced to natural size.

The left third nerve was cut intracranially under nembutal narcosis. One week to two months later the animals were tested with a series of stimuli. Most cats were used repeatedly. In one the right cervical sympathetic was cut in addition to the left third nerve.

Since the pupil, after division of the third nerve, is wide if not maximally dilated, it is unsuited for the study of sympathetic action, but 1 to 2 drops of a 1 per cent eserine solution applied locally cause a constriction of the iris and permit the study of reflex dilatation. The threshold of pupillo-dilator fibers in the cephalad end of the divided cervical sympathetic is extremely low (secondary coil at 13 cm. and tilted to an angle of 80°) and is not altered by eserine, proving that this drug acts exclusively on the iris parasympathetic endings. A dilatation of the eserinized and parasympathectomized eye following a peripheral stimulus indicates sympathetic excitation; absence of such dilatation, when the normal eye

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dilates, indicates that the action is exclusively through inhibition of the tonus of the third nerve. Further experiments were carried out on these cats after metrazol had been given in subconvulsive or convulsive doses, since this drug greatly increases the excitability of the sympathetic centers (Gellhorn and Darrow, 1939).

RESULTS

The cat fastened in the wooden box frequently struggled and such movement was regularly accompanied by a dilatation of the normal or the
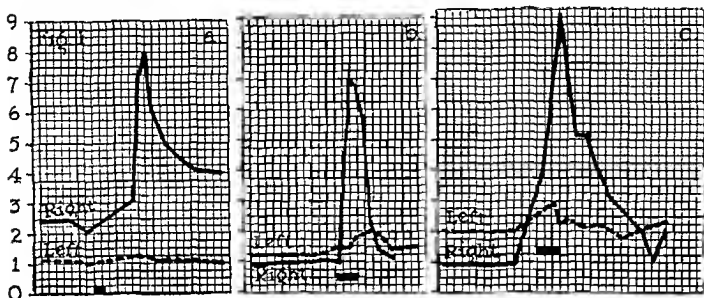
FIG. 1–5. ———— right normal eye; - - - - left eye, the third nerve of which was cut; loca application of 1 per cent eserine.

FIG. 1. Cat, no narcosis. Stimulation of the paw of the left foot with faradic current. Harvard inductorium. 3 volts in the primary circuit. (a) Distance between primary and secondary coil, 6.0 cm.; 5 sec. (b) 5.0 cm.; 10 sec. (c) 3.0 cm.; 20 sec. In all these experiments struggling and vocalization.

sympathectomized pupil, but the parasympathectomized eserinized pupil never changed its size. Stimuli applied to non-narcotized and to etherized cats elicited, not only the pupillary changes to be described, but also general movements and vocalization. The parasympathectomized and eserinized pupil failed to dilate, however, except for a few slight responses (Fig. 1–3). Figure 1a shows the left parasympathectomized pupil to remain unchanged
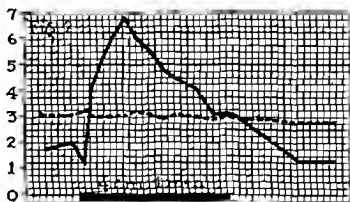
FIG. 2. Cat; deep ether narcosis, no movements, no vocalization. Stimulation of the paw 4 cm. for 60 sec.

while the normal pupil dilates from 2 to 8 mm., on application of the faradic stimulus (5 sec. coil distance 6 cm.). Increase in intensity and duration of the stimulus (10 sec., 5 cm.) leads to less than 1 mm. dilatation on the left side, whereas the normal eye dilates from 1 to 7 mm. (Fig. 1b). Very powerful stimulation (20 sec., 3 cm.) leads to marked struggle and vocalization

and calls forth a large dilatation of the normal pupil but only a feeble one of the parasympathectomized pupil (Fig. 1c). Figure 2 shows complete absence of a sympathetic response to a stimulus which lasted 60 sec. and provoked a marked and prolonged dilatation in the normal eye. Figure 3 shows a maximal dilatation in the normal eye, from 2 to 13 mm., while the para-sympathectomized pupil enlarged from 3 to 4 mm. Even the strongest stimuli produced no dilatation in the parasympathectomized eye in most experiments, and never one greater than 1 mm. (Fig. 1b, 1c, 3). Narcosis did not influence these results.
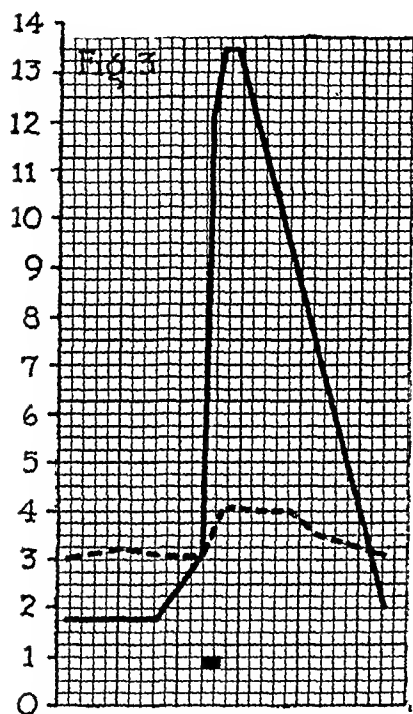
FIG. 3. Cat. Ether narcosis. Sciatic stimulated. 11 cm.; 5 sec.
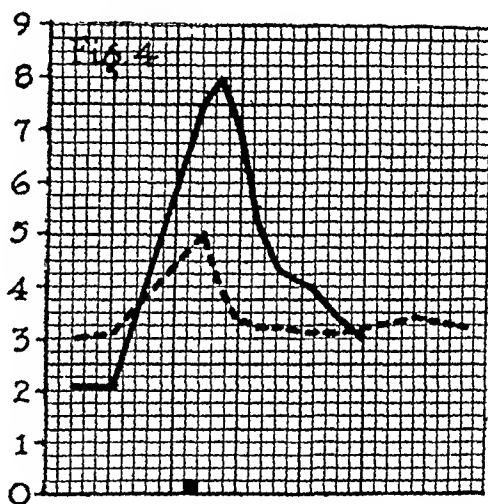
FIG. 4. Stimulation of the sciatic at 4 cm. distance for 3 sec. in light ether narcosis. Some movements and vocalization. The animal was injected with 65 mg. metrazol intraperitoneally prior to the stimulation. No convulsions.

Similar experiments after metrazol administration are illustrated in Fig. 4. In the presence of this drug the parasympathectomized eye responds to sciatic stimulation to a greater extent than in its absence, but the dilatation is still but a small fraction of that shown by the normal eye. When strong sympathetic impulses are present they will cause a dilatation of the parasympathectomized and eserinized pupil. This is shown by pupil changes during metrazol convulsions (Fig. 5). The left eye, which can dilate only by means of sympathetic impulses, increases its diameter to 8 mm., whereas the normal eye dilates maximally, to 13 mm. The rapidity of the changes in both pupils and the quick return to the original diameters makes it highly

improbable that adrenin plays any rôle in these reactions. The second pupillary dilatation (Fig. 5) indicates a second but weaker sympathetic discharge.

In another animal, the left pupil was parasympathectomized and eserinized and the right one sympathectomized. The pupillary dilatation of the left eye during metrazol convulsions was again from 2 to 8 mm., but the sympathectomized pupil dilated only to 7.5 mm. instead of to 13 mm. as did the normal one* (Fig. 6).
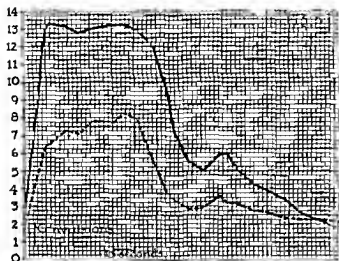
FIG. 5. Cat; no narcosis. Convulsions induced by 70 mg. metrazol intraperitoneally.
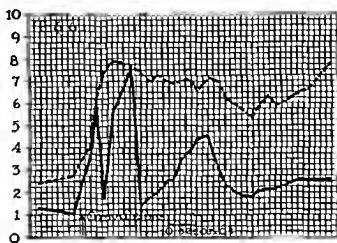
FIG. 6. Cat. ———— right eye; right cervical sympathetic cut. - - - - - - - left eye; left third nerve cut; local application of 1 per cent eserine to left eye. Metrazol convulsions after 80 mg. metrazol intraperitoneally.

These experiments support two conclusions: (i) The difference in pupillary dilatation between the normal and the sympathectomized pupil, in response to stimulation of afferent nerves, is largely due to the tonic action of the sympathetic; (ii) Reflex excitation of the sympathetic plays at most an insignificant rôle.

DISCUSSION

The pupillary dilatation following the stimulation of afferent nerves involves one or several of the following mechanisms: (i) Humoral dilatation (adrenin), (ii) nervous dilatation by, (a) Inhibition of the third nerve nucleus, or (b) Excitation of the cervical sympathetic.

Humoral dilatation can hardly play a rôle in these experiments, since marked dilatation occurs in the normal eye in the absence of a reaction in the parasympathectomized eye, while any humoral effect would be bilateral. Some of the stimuli employed were strong enough to elicit a humoral dilatation of pupils sensitized by previous removal of the superior cervical gan-

* It is interesting to note that the right eye in Fig. 6, which can alter its diameter only by varying the parasympathetic tone, shows, in addition to dilatation (parasympathetic inhibition), brief periods of pupillary constriction (parasympathetic excitation), whereas the left eye shows a continuous sympathetic discharge (cf. Gellhorn and Darrow, 1939).

glion, as shown in other experiments. Moreover, Gellhorn and Darrow (1939) found, in confirmation of the earlier work of McDowall (1925) and Bain, Irving and McSwiney (1935), that removal of the adrenals did not essentially alter the pupillary responses.

The older results in an extensive literature on reflex stimulation of the pupils are frequently difficult to evaluate because quantitative data concerning the mode of stimulation are omitted. The discrepancy between the experiments of Schiff (1875), who reported sensory stimulation without effect on the pupil after sectioning either of the spinal cord or of the cervical sympathetic, and those of Vulpian (1874) and Tuwim (1881), who observed pupillary dilatation after sectioning the cervical sympathetic or removing the superior ganglion, is hard to explain. The majority of investigators seem to agree with Vulpian and Tuwim rather than with Schiff and support the view that the reflex pupillary response is largely due to inhibition of the parasympathetic. Bechterew (1883), particularly, showed that the division of the brain stem behind the superior colliculi eliminates reflex dilatation of the pupil, although the light reflex remains intact, and concluded that pain causes a reflex inhibition of the third nerve center. His observations in man support this interpretation, for in man pain causes reflex dilatation only if the pupils are narrow and then they dilate only to the diameter they would possess in dim light. Braunstein (1894) reports numerous experiments, with quantitative measurements of the pupils, which show that sensory stimulation leads to pupillary dilatation after elimination of the cervical sympathetic but not after severing the spinal cord above the ciliospinal center. Similar results follow stimulation of afferent fibers in the splanchnic, confirmed recently by McDowall (1925), Byrne (1933), and Bain, Irving and McSwiney (1935).

The results of Lieben and Kahn (1930) fit into this interpretation, though the authors assume, without evidence, the additional involvement of a sympathetic center on the floor of the fourth ventricle. However, Chen, Lim, Wang and Yi (1936) showed that stimulation of the "sympathetic" pressor area at the bottom of the fourth ventricle produces, in addition to various sympathetic effects, a dilatation in a sympathectomized pupil only minimally different from that in the normal.

Karplus and Kreidl (1912, 1918) recognized the importance of inhibition of the third nerve in reflex dilatation of the pupil, but attributed considerable importance also to the sympathetic centers. This proof, however, is by no means convincing. They report (1911) one experiment without objective record, with the third nerve cut on the right side and the cervical sympathetic on the left, in which sciatic stimulation produced a moderate dilatation of the parasympathetomized pupil. In view of the observation of ourselves and of v. Brücke (1931) that the parasympathectomized pupil is about maximally dilated,* this statement appears to be erroneous. The later ob-

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* v. Brücke found the diameter varied between 12 and 13 mm. in the cat!

servations of Karplus and Kreidl (1918), that section of the upper cervical cord abolishes pupillary dilatation in response to stimulation of the sciatic or of the branchial plexus but not to stimulation of the trigeminus, require ~~... ... ...~~ probable that the same mechanism hese experiments, and Karplus and Kreidl themselves interpret the effect of the stimulation of the fifth nerve as an inhibition of the third nerve center.

We, therefore, conclude, from a study of the literature and from our own observations, that pain stimuli elicit a dilatation of the pupil mainly by inhibiting the center of the third nerve. With excessive stimuli a small sympathetic discharge may also occur. A few discordant observations in the literature seem to be due to special conditions.

Anderson (1904) noted a pupillary dilatation in the parasympathectomized eye not only after stimulation of the sciatic but also after stroking the skin. We have been unable to confirm this and believe that Anderson, by deep chloroform narcosis, functionally eliminated all higher centers and created (by disinhibition) a state of heightened excitability of the ciliospinal center. This is further suggested by the presence of hippus. Foerster (1936, p. 227) noted an analogous reaction in patients with complete lesions of the cervical cord, in whom passive lifting of the paralyzed arm produced a maximal dilatation of the pupil.

If this interpretation is correct, it is to be expected that pharmacological agents, by heightening the excitability of the ciliospinal center, might bring about a sympathetic reflex dilatation, although this is absent under normal conditions. This seems to be proven by the experiments with metrazol here reported. They show a striking analogy to observations of Luchsinger (1880) and Guillebeau and Luchsinger (1882) who found, in contradistinction to the literature cited earlier, that spinal cord section did not interfere with the reflex dilatation while section of the cervical sympathetic abolished it. Such effects were only obtained, however, when the excitability of the ciliospinal center had been heightened by cocaine, picrotoxin, or strychnine. It is therefore, not permissible to infer from experiments carried out under such conditions that the normal reflex dilatation of the pupil involves the cervical sympathetic. What paths are involved in emotional excitement remains to be seen. The study by Dechaume (1937) of a dog whose thoracic and abdominal spinal cord had been removed seemed to indicate that emotional dilatation is linked up with the integrity of the ciliospinal center.

The slightly greater diameter of the normal than of the sympathectomized pupil* on reflex stimulation is probably due to the tonic action of the sympathetic, which becomes more pronounced as inhibition diminishes the parasympathetic tone. This interpretation seems to agree with that of v. Brücke (1931).

Since the sympathetic pupillary response to pain is ordinarily slight or

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* The difference is ordinarily about 1 to 2 mm.

completely absent, the physiological significance of the sympathetic pupillary fibres must be discussed briefly. Experimental and clinical observations showing a decreased pupillary diameter after elimination of the cervical sympathetic indicate its tonic action. Alteration of this tonic function by pain occurs only when excessive stimuli are used or when a state of increased excitability has been induced in the central nervous system, and particularly in its sympathetic division. But the tonic activity may vary within wide limits, dependent on the intensity of the light reflex, for Gullberg, Olmsted and Wagman (1938) found that the sympathetic dilator tonus increases with increasing dark adaptation.

Under the conditions of reflex stimulation, however, the sympathetic tone of the pupillary fibers remains unchanged, though other sympathetic fibers in the cervical sympathetic may be excited. The contraction of the nictitating membrane, which regularly accompanies pupillary dilatation, is sufficient proof of sympathetic excitation; but this may occur when the normal and sympathectomized pupils dilate to the same extent, *i.e.*, when there is not the slightest indication of an alteration in sympathetic tone in the pupillary fibers.

Conclusions

The rôle of parasympathetic inhibition and that of sympathetic excitation in reflex pupillary dilatation in cats was analyzed by recording the pupillary changes in the normal, the parasympathectomized, and in the sympathectomized eye. It is shown that:

1. After section of the third nerve and local application of eserine, the threshold of the cephalad end of the cervical sympathetic remains unchanged; indicating that such a pupil may be used as an indicator of sympathetic impulses.

2. On reflex stimulation which evokes struggle and vocalization the parasympathectomized pupil remains unchanged or shows a very slight dilatation (not more than 1 mm.), whereas the normal pupil may dilate 7 mm.

3. After sensitization with metrazol the sympathetic reflex response of the pupil increases.

4. During metrazol convulsions the parasympathectomized and eserinized pupil dilates to 7 or 8 mm.

It is concluded that under normal conditions the pupillary dilatation in response to pain is almost exclusively due to parasympathetic inhibition but that under conditions which increase the excitability of the ciliospinal center, sympathetic discharges may contribute materially to the dilatation.

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EFFECTS OF HYPOGLYCEMIA AND PENTOBARBITAL SODIUM ON ELECTRICAL ACTIVITY OF CEREBRAL CORTEX AND HYPOTHALAMUS (DOGS)*

HUDSON HOAGLAND, HAROLD E. HIMWICH, ELDRIDGE
CAMPBELL, JOSEPH F. FAZEKAS AND ZAREH HADIDIAN

*The Biological Laboratories, Clark University, Worcester, Mass., and The
Departments of Physiology and Pharmacology, and Surgery
Albany Medical College, Albany, New York*

A CONSPICUOUS property of most normal human electroencephalograms is the presence of regular sinusoidal alpha waves or "10-per-second" waves, that may best be recorded from the resting subject with closed eyes. Hoagland and his collaborators have demonstrated that the *relative frequency* of these waves, *other things being equal*, is proportional to the rate of respiration of the cortical cells producing the waves. Changes of frequency produced by temperature via diathermy (Hoagland, 1936a, 1936b) give definite energies of activation or temperature characteristics identical with the principal modal values for oxygen consumption and CO_2 production of numerous tissues *in vitro*. This, from the nature of the equation used, could only occur if frequency were proportional to respiration rate. Dinitrophenol accelerates alpha frequencies along a smooth curve (Hoagland, Rubin and Cameron, 1939) and thyroxin also accelerates the frequencies along with the B.M.R. (Rubin, Cohen and Hoagland, 1937). Reduction of blood sugar by insulin causes the alpha waves to slow, after a lag, with the falling sugar and later to return when glucose is given (Hoagland, Rubin and Cameron, 1937). Himwich *et al.* (1939) have confirmed this last finding and demonstrated a relation between oxygen consumption of the brain and alpha wave frequency.[1] Sugar is probably the chief brain fuel (Himwich and Nahum, 1932).

These findings are not, of course, to be interpreted as meaning that the *only* modifiers of brain wave frequencies are necessarily changes in cell respiration. Afferent stimulation, which modifies frequencies, may do so by locally changing cell respiration or by modifying the electrical constants (impedance) of the cells or their connections through permeability changes. Absolute differences in frequencies of different cell groups also are not to be regarded as due to corresponding absolute differences in rate of O_2 consumption, but rather to differences in structure of the cell walls or connecting fibers. These considerations in no way militate against the view that certain *relative* frequency changes under standard conditions are due to changes in rates of cell metabolism. The waves may be regarded as "spontaneous" in

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[1] These rate considerations apply only to frequencies which have the dimensions of rates, *i.e.*, reciprocal times. Amplitude and total energy are not rates and their relation to metabolism should not be confused with the frequency factor.

origin, or due to closed or "reverberating" circuits set off initially by afferent stimuli. In either mechanism a type of relaxation oscillator system discussed by Hoagland (1936b) is equally consistent with the experimental findings. In the latter case oxidative recovery from the refractory state of the circuit may be pictured as determining the frequency.

Page (1937) in a review has written, "Important results of Quastel and Wheatley, and Davis and Quastel seem to show that all narcotic drugs (including the barbiturates) have one property in common: they inhibit specifically at low concentration the oxidation by brain of substances essential to the metabolism of carbohydrates such as glucose, lactic acid and pyruvic acid. . . . Their results suggest that narcotics do not interfere with access of oxygen to brain cells or with activation of oxygen by brain catalysts, but with the mechanisms that result in activation of lactic acid and pyruvic acid."

An anesthetic like pentobarbitol sodium (nembutal), on the basis of this *in vitro* work with brain slices, might give typical brain wave changes characteristic of reduced brain respiration. As in hypoglycemia, where respiration is slowed by removing the brain's fuel supply, so we might expect that nembutal would (i) slow primary or "alpha" frequencies and (ii) as in hypoglycemia, delta waves (all waves longer than the basic alpha waves) might become more pronounced. Deprivation of O_2 by N_2 breathing in man in extremes of deprivation slows the alpha frequency and, in lesser degrees of deprivation, increases the random slow wave activity (Gibbs, Davis and Lennox, 1935). Variations in pH due to CO_2 changes produce in themselves modifications. Increase in CO_2 enhances short wave components (Lennox, Gibbs and Gibbs, 1936). These are complicating factors in considering alpha frequencies directly in relation to oxygen tension. But the evidence, from the temperature analysis, that cell respiration is a basic determinant of frequency is not subject to criticism on pH grounds. The energies of activation for alpha frequencies not only agree with the principal modal values for cellular respiration, but Hadidian and Hoagland (unpublished) have been able to show that two of the three values correspond to specific respiratory enzyme systems which, under the conditions of the experiments, act as chemical pacemakers for the cortical respiration. Sizer (1937) has shown that pH variations over a wide range (3.2–7.9) have no effect on activation energies of certain specific enzymes.

Prawdicz-Neminski (1925) showed that the dog cortex displays a basic predominant rhythm of 11 to 16 per sec. along with a secondary rhythm of 20 to 32 per sec. In general we have confirmed his findings. In 18 dogs we have found (i) a basic rhythm presumably corresponding to the alpha rhythm of man ranging from 14 to 18 per sec. in dogs without anesthetic from widely varied parts of the cortical dura, along with faster rhythms of lesser amplitude. The present investigation presents certain information concerning the relative effects of nembutal and of hypoglycemia on the electrical activity of the cerebral cortex and the hypothalamus.

Our records have been taken with either a one-channel magnetic ink-writer recording on paper tape (built by Garceau) or a two-channel matched amplifier system also recording with pens (built by Grass). We have used throughout monopolar (grid to ground) recording which has been demonstrated to give better local representation than bipolar recording (Rubin, 1938). The leads were either chlorided silver wires or small pointed solder tips attached to copper wires insulated to 1 mm. or so of the tip. In general a day before the time of observation the dogs were anesthetized with nembutal (0.7 cc. of 5 per cent solution per kg. body weight). The hypothalamus was then exposed surgically under sterile conditions, using the subtemporal route, and leads were inserted. One input grid lead was placed in the region of the mammillary bodies, a second in the region immediately above the optic chiasm and a third on the cortex. A fourth "indifferent" lead was inserted under the scalp behind the homolateral ear. By the next day the dogs were able to walk about and appeared to be well recovered from operation and anesthesia. Four of the 18 dogs were studied as acute preparations under nembutal anesthesia with only the cerebral cortex exposed.

In the figures we refer to responses from "mammillary bodies" and "supraoptic nucleus." It is understood that these structures were located by landmarks at the time of operative exposure and were always checked post mortem, although brain sections were not made. It might thus be more correct to refer to "mammillary body region" and "supraoptic nucleus region."

RESULTS

Figure 1A shows electrical responses from a dog (cortex only) anesthetized with nembutal. At 9:25 the record consists of large delta waves and of a basic rhythm of 10 per sec. This rhythm may be seen to speed up during the day as the anesthetic wears off and, with this increase in "alpha" frequency, there is a decrease in delta wave activity, until at 2:15, when the dog is fully recovered, there is a clear basic "alpha" rhythm of 18 per sec. A second dose of nembutal reëstablishes the earlier condition. The parallelism between the nembutal effect on brain waves and that of hypoglycemia in man is indeed striking (see Hoagland, Rubin and Cameron, 1937), and is what would be expected if the changes in both cases were due to inhibitory action on the respiration of the cortical cells.

Figure 1B shows responses from the unanesthetized cortex of a dog on successive days corresponding to different blood sugar levels. The basic rhythm is reduced from 15 to 8 on the second day with reduced blood sugar and the delta waves are enhanced. This picture is the same as that encountered in the human E.E.G. following reduction of blood sugar by insulin as originally described by Hoagland, Cameron and Rubin (1936, 1937) and confirmed by Yeager and Baldes (1937) and by Himwich *et al.* (1939). Since nembutal and hypoglycemia both affect the electrogram in much the same way, it is obvious that nothing quantitative can be said about changes due to either factor alone in studies of insulin shock which involves the use of nembutal anesthesia.

Figure 1C shows simultaneously recorded responses from anterior hypothalamus and cortex before anesthesia on the day after operation and again 20 min. after an anesthetizing injection of nembutal. The basic cortical ("alpha") rhythm is slowed from 17 to 9, and, as also in Fig. 1A, the amplitude is increased (cf. Derbyshire, Rempel, Forbes and Lambert, 1936). No significant effect is seen upon the hypothalamic lead. This was typical in all

Fig 1a

Fig 1b

Fig 1c
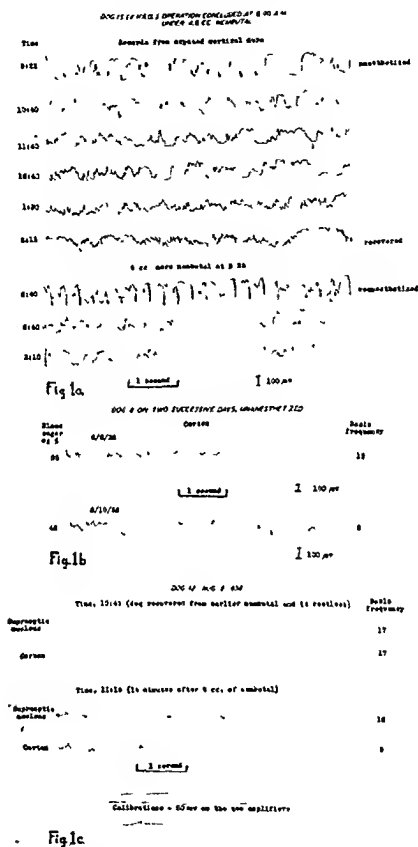
FIG. 1. A. Sample records showing effect of nembutal anesthesia on the dog cortex. The dog is fully recovered from its first dose by 2:15. Note the decrease in delta waves and increase in the basic "alpha" frequency with recovery. Note also the spike and wave formations following the second dose. Negativity is up throughout.

B. Effect on "alpha" rhythms of two blood sugar levels. This dog was operated on Aug. 8, 1938.

C. Two pairs of simultaneous records showing effect of nembutal on cortical waves and lack of effect on waves from the anterior hypothalamus. Dog operated on Aug. 15, 1938.

14 dogs. Surgical anesthesia with nembutal has no effect on the electrogram from either anterior or posterior hypothalamus.

Grinker (1937) described an ingenious method of recording from the hypothalamic region by embedding a grid lead in the bone in the roof of the pharynx below the hypothalamus. His method is applicable not only to common laboratory mammals but also to man. Hoagland, Cameron, Rubin, and Tegelberg (1938), using Grinker's method, have made studies in man of simultaneously recorded cortical potentials and potentials from a lead in the pharyngeal roof near the hypothalamus. Asynchronous alpha waves were found at both leads and, following emotionally charged remarks and queries, delta waves were obtained at both leads. A month later, and quite inde-
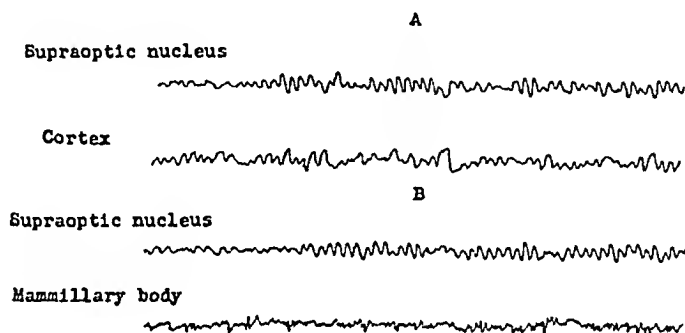
FIG. 2. Two pairs of records from an unanesthetized dog. A. Upper, from anterior hypothalamus; lower, from cortex (simultaneous recording).

B. Upper, from anterior hypothalamus; lower, from mammillary body (simultaneous recording). The basic rhythms are 14 per sec.

pendently, Grinker and Serota (1938) reported this same result. Hoagland, Cameron and Rubin showed that the hypothalamic delta waves on the average precede the corresponding cortical deltas by approximately 4 msec., suggesting detonation of the cortex by way of the hypothalamus following emotional stimulation. In view of this it was desirable to compare responses obtained directly from the anterior hypothalamus in unanesthetized animals with those obtained by the Grinker technic in which the hypothalamic lead is embedded in the bone. An especially clear-cut record of this sort on an unanesthetized dog is shown in Fig. 2. Simultaneously recorded, 14 per sec. nonsynchronous, basic or "alpha" rhythms are seen from cortex and anterior hypothalamus which are like those obtained by the Grinker technic in man (cf. figures in Hoagland, Cameron, Rubin and Tegelberg, 1938 and Grinker and Serota, 1938). The posterior hypothalamic lead in the mammillary body region of this dog, though only about 1 cm. distant, shows an entirely independent and faster rhythm, thus confirming the localizing efficacy of our method of monopolar recording.

Figure 3 demonstrates the changes in activity of cortex and anterior and posterior hypothalamus during insulin shock in a dog initially anesthe-

tized with nembutal. Since from 10:05 to 2:30 the nembutal effect is wearing off while the hypoglycemic effect is coming on, we should expect the cortical picture to be relatively constant since hypoglycemia and nembutal both slow "alpha" waves and enhance delta wave activity (Fig. 1). This is seen to be the case. By 2:30 may be seen the typical picture of hypoglycemia familiar in man for a corresponding blood sugar level. There is at this time comparatively little hypothalamic change. By 5:00 the cortical activity is
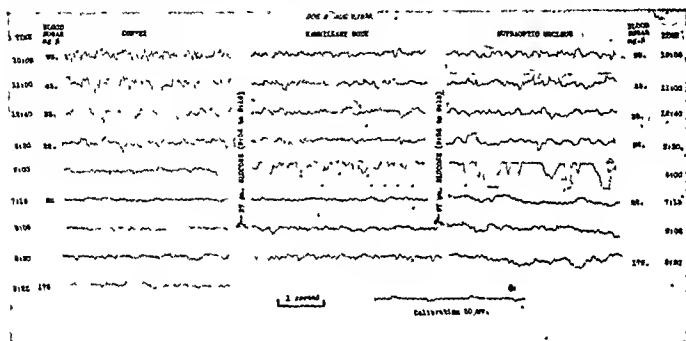
FIG. 3. Records from cortex, posterior hypothalamus, and anterior hypothalamus during insulin shock and recovery. At each recorded time successive records were made from the three regions about one minute apart. The dog was given nembutal at 9.10 and again at 11:50. The frequency changes in "alpha" waves cannot, therefore, be related to sugar changes alone. Note augmentation of cortical delta waves at 2.30 and flattening of record by 7:15. Note slowness of return of cortical activity after administering sugar. The hypothalamic responses are much less depressed and return more rapidly after sugar injection (see records of 9:06 and 9.20). Note the violent responses of the hypothalamus at 5:00, producing amplifier block. These surges were not accompanied by convulsions nor were they associated with pulse or respiration. Operation on Aug. 8, 1938.

greatly reduced but there are violent surges of activity at the hypothalamic lead. The blood sugar had been 45 mg. per cent or less for 6 hours. By 7:15 the cortex is silent but there are still regular rhythms from the hypothalamus, especially from the mammillary body. After injection of glucose at 9:04 the amplitude of hypothalamic waves increases rapidly, the cortical response remaining feeble and irregular. This result is typical of 5 experiments on as many dogs.

Figure 4 shows the great stability of the hypothalamus during insulin shock. The blood sugar ranged around 35 mg. per cent for 5.5 hours but the basic rhythms at the two hypothalamic leads, simultaneously recorded, are unaffected. The regular large deflections especially in evidence at 4:30 from the mammillary body are believed to be electrode artefacts.

Figure 5 shows clearly the early failure of the cortex with pronounced
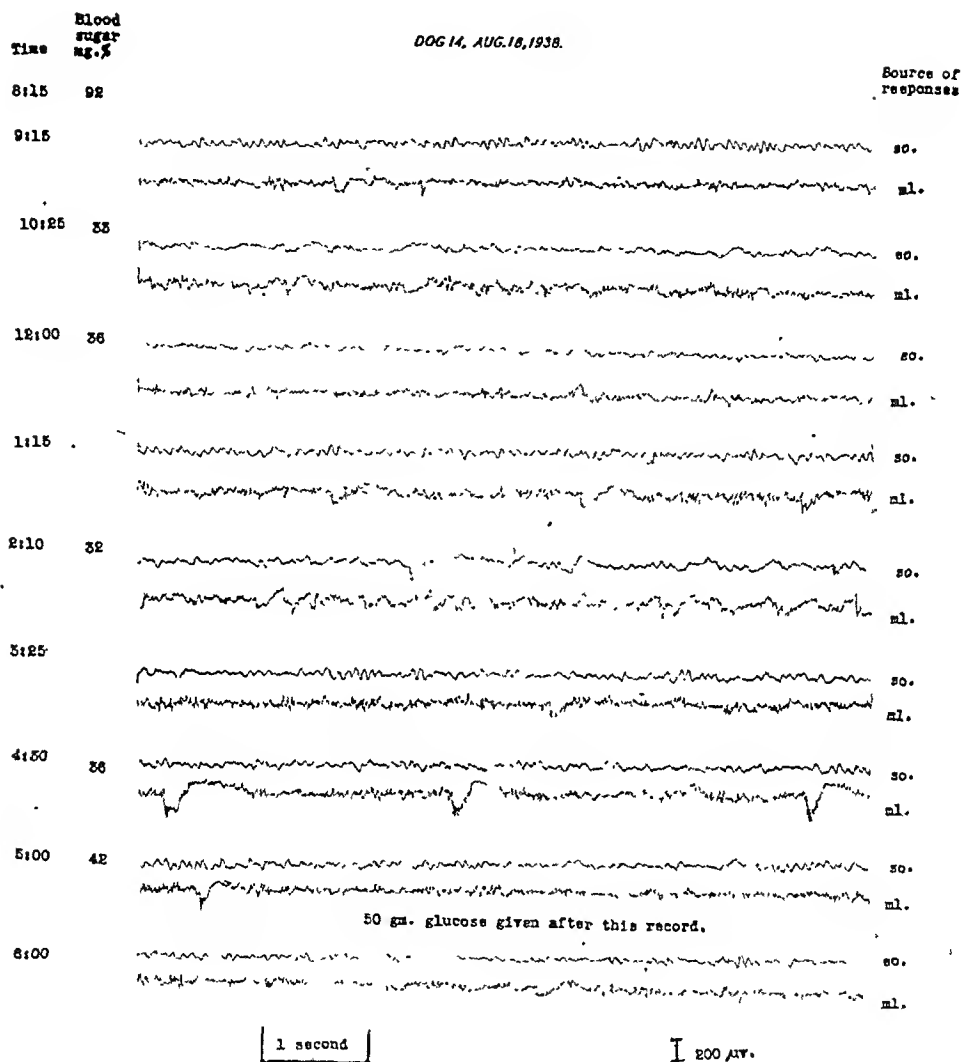
FIG. 4. Simultaneously recorded pairs of electrograms taken from the region of the supraoptic nucleus (so.) and from the region of the mammillary body (ml.) during the lowering of blood sugar by insulin. 20 U. insulin were given repeatedly at 8:15, 10:15, 12:10, 2:35, and 4:35. The first record at 9:15 is prior to anesthesia (the dog was operated on the day before). 4 cc. nembutal were given at 10:15. At 5:35 glucose (50 gm.) was given. Note the great stability of responses from the hypothalamus. Despite anesthesia and blood sugar changes the activity of this center is essentially unmodified.

hypoglycemia in an anesthetized dog, along with the relative stability of the hypothalamic activity. At 5:55 the record shows a uniform rhythm from the hypothalamus which in 3 animals we observed very late in hypoglycemic
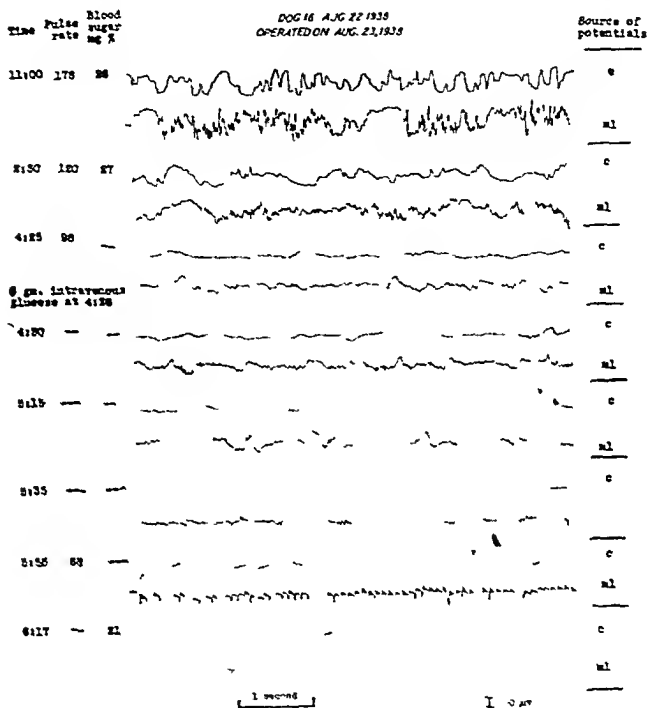
FIG 5 Showing the effect of prolonged insulin hypoglycemia on simultaneously recorded oscillograms from the cortex (c) and from the mammillary body region of the hypothalamus (ml) For seven hours the blood sugar analyses are not >27 mg per cent A small amount of glucose at 4 28 has little if any restorative action Note failure of cortex and relative stability of hypothalamic activity and also progressive fall in pulse rate Note the rhythms from the hypothalamus at 5 55 prior to complete failure of activity at the two leads These rhythms did not correspond to pulse or to respiration or to any detected movement of the animal At 6 20 the brain was frozen and found to contain only 14/100 gm of glycogen

shock Because of restlessness and hypoglycemic convulsions producing artefacts and endangering the electrodes, we used anesthesia (nembutal) in
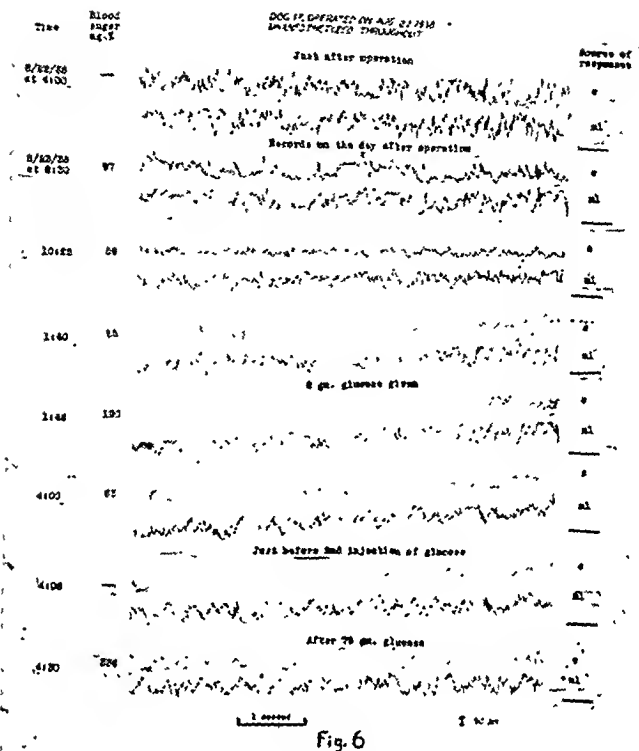
Fig. 6
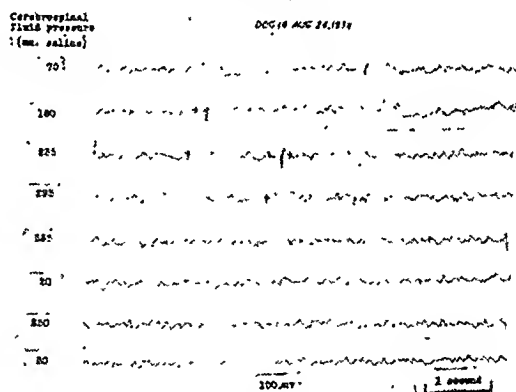
Fig. 7

FIG. 6. Records from an unanesthetized dog showing cortical (c) responses and responses from the mammillary body region of the hypothalamus during insulin hypoglycemia and recovery. Sugar was given between the 1:40 and 1:46 records, followed again by insulin. Sugar was again given after the 4:08 record. The blood sugars in this experiment remained comparatively high. This particular dog, like some persons, showed irregular waves difficult to quantify.

FIG. 7. The cerebrospinal fluid pressure was changed by running saline in and out of the cisterna magna. No correlating modifications of the cortical electrogram recorded through the dura were seen. Nembutal anesthesia.

nearly all our insulin experiments. One of our dogs was, however, of so quiet a temperament that we were able to experiment post-operatively without using anesthesia. Unfortunately, this dog, like some persons, did not have a very clear "alpha" rhythm and showed considerable delta wave activity. Figure 6 shows results of this experiment. The blood sugar was not reduced below 55 mg. per cent and the corresponding small changes in cortical activity and lack of changes in hypothalamic activity are of the sort to be anticipated from the above discussion and also from the work with man.

Marked hypoglycemia raises the intracranial pressure about 50 per cent (see Gellhorn, 1938) and it was possible that some of the electroencephalographic changes accompanying hypoglycemia might be due to hydrostatic pressure effects. Walter (1938) in a review notes that with the elevation of intracranial pressure resulting from such agencies as ventricular obstruction, concussion, meningitis and cerebral tumor that delta waves at about 3 per sec. occur over the entire cortex. But no correlation has been found between actual cerebrospinal fluid pressure and the slow waves. Rather, the delta waves seemed to be associated with the extent of cortical impairment of function. He reported, however, that the generalized delta activity could be reduced by intravenous injections of hypertonic solutions but it was little affected by the removal of cerebrospinal fluid. Walter has described cases of cerebral oedema with no measurable pressure changes which showed the slow discharge. The oedema seemed to be the important factor rather than the hydrostatic pressure.

To test the effect of hydrostatic pressure *per se*, we inserted into the cisterna magna a hypodermic needle which was held rigidly fastened to a nail parallel to the needle and driven into the occipital bone just above the foramen magnum. Various heads of hydrostatic pressure (saline solution) were then applied from a reservoir and measured on a manometer. Figure 7 shows the results. It is clear that a 16-fold variation in pressure in itself produces no effect whatever on the cortical electrogram of the dog, at least during the hour or so consumed by the experiment.

DISCUSSION

These results with dogs confirm and extend the findings of Hoagland, Rubin and Cameron concerning hypoglycemia and the electroencephalogram in man. Along with the nembutal effects they add further evidence in favor of the view that the relative *frequency* of cortical rhythms is determined basically by the respiratory rate of the cortical cells.

Dubner (1938) in a preliminary abstract reports studies of the electrical activity from the lateral geniculate body of cats under nembutal anesthesia. He writes, "Insulin (up to 15 units per kgm. subcutaneously) did not give gross convulsions, though potential changes were definite within an hour. The small high-frequency potentials superposed on the usual optic rhythm were accelerated and intensified; and the normal 'on' response to light was replaced by a series of 3 to 5 large oscillations, mainly positive, and followed

by a prolonged high frequency after discharge. . . . The normal picture was restored within five minutes after intravenous injection of glucose." These results showing an increase in fast wave components with hypoglycemia appear to be at variance with our findings with dogs and with the results obtained with man. Different animals and different centers were involved in Dubner's experiments as compared to ours. Also, one is led to wonder if the high frequency components may not be new superimposed rhythms, and not progressive *frequency* accelerations in the same rhythm. Further, nothing is said concerning modifications of the frequency of the basic optic rhythm. If the nembutal effect were wearing off as the hypoglycemic effect were coming on we would expect that the basic optic rhythm to which he refers would remain unchanged as in our Fig. 3 for reasons already pointed out. A more extended discussion of this matter would not appear to be profitable until Dubner's results are published.

Sugar and Gerard (1938) have shown that following sudden occlusion of the circulation that the "highest" and phylogenetically newest centers fail in electrical activity first and regain activity last when the circulation is restored. Their records were made on anesthetized cats, using the Horsley-Clark technic, but did not include the hypothalamus. Following occlusion they found, in general, that centers were first briefly stimulated to hyperactivity, followed by a decrease in both amplitude and frequency of the waves. Our results showing greater resistance to hypoglycemia and nembutal of the hypothalamus in contrast to the cortex are similar to their findings on the susceptibility of new and older centers to oxygen deprivation.

We find that only in very late stages of prolonged hypoglycemia in dogs does hyperactivity of the hypothalamus occur (Fig. 3 and 5). This hyperactivity is manifested only a long time after cortical failure occurs, an advanced condition which in human insulin treatments does not take place. Hyperactivity of the autonomic system, as judged by sweating, salivation, etc. in both man and dogs occurs much earlier, at a time when the cortical response shows either a greatly slowed or absent alpha rhythm along with prominent delta waves. In all of our experiments, the dog hypothalamic responses have at this time been quite unchanged. It seems probable from these findings that the relatively early external signs of sympathetic hyperactivity result from a reduction of cortical control over sympathetic motor mechanisms which permit more extensive motor manifestations for the *same* degree of hypothalamic discharge rather than from a direct stimulating action at this stage of hypoglycemia on the hypothalamus. Hyperactivity of this center does occur but only in late stages of prolonged hypoglycemia.

Our findings superficially appear to be at variance in one respect with a published figure from Grinker and Serota (1938) who show a slowing of the hypothalamic rhythm in the cat *immediately* following the injection of nembutal. This result may be due to one of several reasons (i) the cat and dog may differ in this respect, though this is unlikely, (ii) our experiments are

not exactly comparable since we have never recorded hypothalamic changes *immediately* after injecting nembutal but have made such comparisons only before and after an anesthetizing dose 20 min. or so following the injections, (iii) the dose used by Grinker and Serota was much greater than ours, since they superimposed on the original anesthetizing intraperitoneal dose 100 mg. of nembutal intravenously, an amount which by itself would be more than enough to anesthetize a two kilogram cat. With such an extreme depression of the c.n.s. we would expect hypothalamic activity to decrease as it does, for example, in the extremes of hypoglycemia. Our experiments indicate that as far as anesthetic amounts of nembutal are concerned, cortical activity is depressed, while hypothalamic activity seems unaffected.

SUMMARY

1. Electrograms have been recorded from the dog cortex (18 dogs) and from anterior and posterior hypothalamus (14 dogs) both with and without anesthetic under varying conditions of insulin hypoglycemia.

2. The effect of hypoglycemia on the cortical activity is similar to that in man, the alpha rhythms are slowed and the delta waves increase in prominence. In more prolonged hypoglycemia the cortical responses fail completely and are restored only an hour or so after injection of glucose. Both posterior and anterior hypothalamus show much stability compared to the cortex. No changes are noted until some time after failure of electrical activity of the cortex when a short period of hyperactivity of the hypothalamic centers may develop, followed later by failure just before death. Injected glucose restores hypothalamic activity some time before that of the cortex.

3. Nembutal, which reduces cortical respiration *in vitro*, produces cortical changes similar to those of hypoglycemia except that it considerably enhances the amplitude of alpha waves at the same time that it slows their rhythm. In contrast to this, no differences were seen in hypothalamic rhythms before and after nembutal anesthesia.

4. Our results show independence of responses from cortex, from a region near the supraoptic nucleus, and from the mammillary body region. The latter two grid leads were approximately 1 cm. apart. The anterior hypothalamic response, recorded directly by our method, gives records indistinguishable from those obtained by the Grinker technic in which a grid lead is embedded in the bone at the roof of the pharynx.

5. Changes (as much as 16-fold) in hydrostatic pressure of the cerebrospinal fluid are *per se*, without effect on the cortical electrogram.

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CORTICAL ACTION POTENTIALS DURING ANESTHESIA

H. K. BEECHER AND F. K. McDONOUGH

Surgical Laboratory, Massachusetts General Hospital and Department of Physiology, Harvard Medical School, Boston

INVESTIGATION of the anesthesia process has been handicapped by lack of precisely measurable elements. The electrical activity of the central nervous system, and in particular that of the cerebral cortex, offers a measurable component which can be followed during anesthesia. With the rapid development of electrophysiology in recent years, new tools have been evolved which promise to be of great service in clarifying certain phases of the action of anesthetic agents The use of the electroencephalogram as an approach to the study of anesthetic agents is not new,* but the usefulness of the results obtained has been limited by the fact that generally each observer has studied only one or two agents. Even when the same agents have been studied in several laboratories many other factors have varied, such as animals, position and type of electrodes, depth of anesthesia, and so on It is impossible to consider these data from a comparative standpoint. The study of widely differing anesthetics under comparable conditions was begun by Derbyshire, Rempel, Forbes and Lambert (1936), who selected three agents as representative of three distinct classes and compared the electroencephalogram under them with that in unanesthetized animals both waking and sleeping. At the suggestion of Dr. Forbes this earlier study has been extended and a considerable number of anesthetic agents has been studied under both light and deep anesthesia, with the aim in view of getting more insight into the nature of the cortical action potentials, and with the hope that leads might be opened up which would throw light on the anesthesia process itself.

MATERIALS, APPARATUS AND METHODS

Animals Cats were employed in all experiments The right posterior sigmoid gyrus of the cortex was exposed, and activity recorded from the region of the sensory area in all cases

Anesthetic agents The seventeen compounds studied and the number of experiments under each follow. Nitrous oxide, 4, cyclopropane, 3, ether, 4, divinyl ether, 4, ethylene, 3, trichlorethylene, 3, ethyl alcohol, 3, ethyl chloride, 3, chloroform, 5, amylene hydrate,** 3, tribromethanol,** 3, "evipal" (1-methyl-5-Δ' cyclohexenyl-5-methyl barbituric acid), 3, paraldehyde, 3, sodium barbital, 3, chloralosane†, 4; and "nembutal" (sodium ethyl (1-methyl-butyl)barbiturate), 3

Levels of anesthesia These were determined by the flexion reflex evoked by electrical stimulation of the central end of the cut left sciatic nerve The stimulating electrodes were

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* For bibliography see Berger, 1929, 1930, Fischer, 1932, Kornmuller, 1935, Gibbs, Davis and Lennox, 1935, Jasper and Andrews, 1936, Gerard, Marshall and Saul, 1936, and others

** Kindly supplied by the Winthrop Chemical Company.

† Kindly supplied by Dr R C de Bodo

silver wires encased in a rubber tube. The cut sciatic nerve was inserted into this tube. These electrodes were connected with the secondary coil of a Harvard inductorium. In circuit with the primary (activated by a 1.5 volt dry cell) was a hand operated mercury contact key and a string galvanometer signal device. The stimuli were make and break shocks spaced from one to three seconds apart, and usually followed by a rapid series of six or eight shocks (by hand). The ipsilateral nerves to the hamstring muscles were left intact so that a flexor response of the lower leg might occur and be recorded on a smoked drum. The strength of the stimulating current was adjusted to give approximately a maximal response. Two levels of anesthesia were arbitrarily chosen and studied in detail: (a) The lightest anesthesia it was possible to work with without producing a disturbing generalized muscular response, on stimulating the sciatic nerve, and (b) The level at which the flexion reflex just disappeared. These two levels cover a wide anesthesia range; the former represents the surgical stage (stage III, plane 1) of anesthesia, while the latter represents deep surgical (stage III, plane 3) anesthesia. For brevity, hereafter, these two levels will be referred to as light and deep anesthesia.

Electrodes. The concentric electrodes described by Beecher, McDonough, and Forbes (1938) were used. These are made of silver supported on a hard rubber base. The grid lead is spike shaped and protrudes 2 mm. beyond the base. It is everywhere insulated except at its tip. At the base it is surrounded by the ground lead, a circular band 1 mm. in width and 6 mm. in outside diameter. The electrodes were freshly chlorided electrolytically each time before use. With these, potential differences were recorded between the surface of the cortex and 2 mm. deep in the interior. When these electrodes are used the ground lead fills a large part of the opening in the skull made necessary in order to identify the desired cortical position. Herniation of the brain is thus in large measure prevented and the arterial and respiratory pulsations minimized. The potential difference appears to be somewhat greater when led off in this manner (interior to surface) than when both leads, though the same distance apart, are applied to the surface. The increased potentials tend to give a clearer recording of changes.

Amplifier. The potential differences between the grid and ground leads were amplified by the direct coupled apparatus described by Forbes and Grass (1937) and were recorded on film with a Hindle string galvanometer. Ten milliseconds units were recorded by a timer on one margin of the film. The signal device recorded the stimuli on the opposite margin.

Results

Frequency and voltage. The standard deviation of the mean* frequency has been calculated in all cases, and is invariably given. It, however, does not show the constancy of the frequency counts from experiment to experiment. The striking constancy of this is shown only in detailed tables which, to conserve space, have not been presented here.

In the case of nitrous oxide, data are given only for light anesthesia, for at normal atmospheric pressures deep anesthesia under this agent is of course impossible without severe anoxia of the tissues accompanying the anesthesia. In all cases care was taken to avoid anoxia.

When frequencies per sec. of the cortical action waves are given, the counts have been made by two observers in all cases, and the two results averaged. They rarely differed by more than two or three waves per sec. Every deflection was counted as a wave. Care was taken to avoid 60-cycle

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* This is derived by the standard statistical formulae:

$$\text{S. D. (individual)} = \sqrt{\frac{\Sigma D^2}{N-1}} = x; \quad \text{S. D. (mean)} = \frac{x}{\sqrt{N}}$$

To test for significant difference: $M_1 - M_2 > 2\sqrt{\overline{\text{S.D.}}^2_{m1} + \overline{\text{S.D.}}^2_{m2}}$

interference In each observation activity was usually recorded continuously
for 5 to 10 sec. A full second was counted in each case, the seconds, though
arbitrarily chosen for counting, were spread out as well as possible over the
entire experiment at the given level of anesthesia. Unless otherwise specified,
the frequencies listed indicate the total count for all discernible waves ir-
respective of type. On the average, 38 counts were made in duplicate for

Table 1 *† *Mean frequency per second of cortical waves during anesthesia*
(With Standard Deviations)

Agent	Light Anesthesia		Deep Anesthesia	
		Including Occasional Large Waves		Including Occasional Large Waves
Nitrous oxide	43 2 ± 0 7			
Cyclopropane	42 9 ± 0 6		42 0 ± 0 7	
Ether	39 9 ± 0 4		38 5 ± 0 4	
Divinyl ether	37 1 ± 0 5		34 9 ± 0 6	
Ethylene	36 7 ± 0 4		37 2 ± 0 5	
Trichlorethylene	33 3 ± 0 5		32 7 ± 0 4	
Ethyl alcohol	31 3 ± 0 5		30 9 ± 0 3	
Ethyl chloride	30 5 ± 0 5		28 8 ± 0 4	
Ethyl urethane	30 4 ± 0 4		29 4 ± 0 3	
Chloroform	28 7 ± 0 3		26 2 ± 0 5	
Amylene hydrate	26 5 ± 0 5		24 9 ± 0 4	
Tribromethanol	22 2 ± 0 6	27 0 ± 0 6	22 3 ± 0 5	22 9 ± 1 0
"Evipal"	21 6 ± 0 7	22 1 ± 0 9	18 3 ± 0 7	18 2 ± 0 8
Paraldehyde	19 8 ± 0 3	22 2 ± 0 6	21 0 ± 0 4	22 3 ± 0 6
Sodium Barbital	18 7 ± 0 4	20 5 ± 0 9	17 7 ± 0 4	18 3 ± 0 9
Chloralosane	13 6 ± 0 3	14 2 ± 0 3	13 3 ± 0 4	13 6 ± 0 4
"Nembutal"	13 5 ± 0 4	15 2 ± 0 4	13 6 ± 0 7	15 3 ± 0 8

* Under several agents bursts of cortical activity occur These bursts appear about
every three to five seconds and are followed by relatively quiescent periods The agents
which show this phenomenon and the frequency per second of the large waves during such
periods of activity, are as follows Tribromethanol, 12, "evipal," 9, paraldehyde, 5, sodium
barbital, 8, "nembutal," 8. (The frequency is the same during both light and deep anes-
thesia)

† D........................ ....orted a faster frequency for "nembutal" than we have
found .... additional experiments were performed These experi-
ments .... (above) furnished counts agreeing with those presented
in Table 1 Apparently the figure given by Derbyshire represents a maximum frequency
whereas ours is based upon the average count for an entire second.

each level of anesthesia for each agent, and 22 counts were made in duplicate
for each experiment with each agent, 11 being under light anesthesia and 11
under deep. For those agents which give rise to bursts of large waves, in
addition to the small ones, the means and their standard deviations have
been calculated not only for the *total* wave count but also for the small waves
alone, omitting the large waves of the bursts in this particular case. The
frequency of the large waves *during* the bursts has also been determined for
those agents which show the phenomenon. "Small" waves refers to the
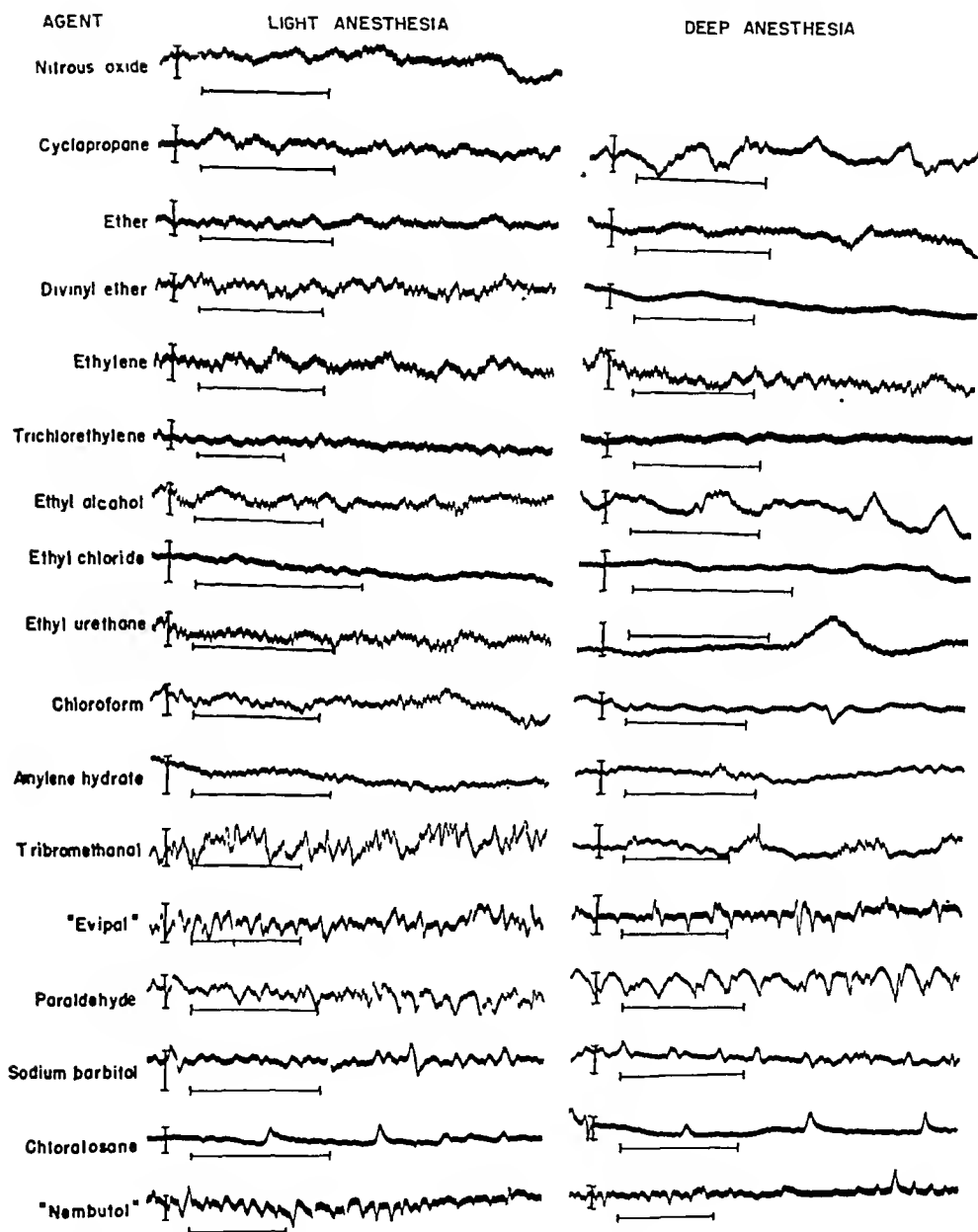
FIG. 1. Typical records of cortical action potentials obtained under each of the agents studied at both light and deep levels of anesthesia. An upward excursion signifies negative potential of the grid lead. This was always the central electrode which penetrated the cortex. Read from left to right. The vertical line (sensitivity) in each case represents 200 μV, and the horizontal line 1 sec.

typical, regular fine waves as distinguished from the less frequent bursts of
very large waves. The voltages for these are given in the Table 2, which
follows. The mean frequencies per second with their standard deviations are
presented in Table 1 and Fig. 2.

The data have further been analyzed as to voltage and frequency of the
waves for both light and deep anesthesia before and after sciatic stimulation.
The entire mass of data has not been analyzed as to the effect of sciatic
stimulation on the voltage of the cortical waves. One experiment for each

Table 2 Effects of sciatic stimulation on the voltage of cortical waves (microvolts)
during anesthesia*

Agent	Voltage— Light Anesth		Voltage— Deep Anesth		Volt Occasional Large Waves Light Anesth		Volt Occasional Large Waves Deep Anesth	
	Before Stim	After Stim	Before Stim	After Stim	Before Stim	After Stim	Before Stim	After Stim
Nitrous oxide	21	46						
Cyclopropane	30	49	22	23				
Ether	24	48	18	17				
Divinyl ether	10	15	9	9				
Ethylene	14	23	9	7				
Trichlorethylene	17	37	13	12				
Ethyl alcohol	18	43	15	15				
Ethyl chloride	15	24	4	5				
Ethyl urethane	28	41	22	20				
Chloroform	20	41	15	14				
Amylene hydrate	18	27	14	15				
Tribromethanol	34	34	15	14	147	152	114	105
"Evipal"	22	21	13	13	86	83	43	45
Paraldehyde	28	28	16	14	94	92	84	75
Sod barbital	19	17	12	14	92	95	206	213
Chloralosane	15	15	10	10	87	85	97	106
"Nembutal"	17	17	12	9	69	71	51	47

* Average, see text

agent has been considered. In measuring voltages the amplitude of 4 to 6
typical blocks (strips of film 1 to 3 cm. in length) have been measured where
possible. These 4 to 6 values have been averaged and are presented in Table
2 and in Fig. 3. Figure 3 does not include the large waves. Stimulation had
no effect on their voltage, in the cases studied.

The mean frequencies of the cortical waves before and after sciatic
stimulation are presented in Table 3. This division of the data increases the
error inherent in the mean values as a result of the smaller number of in-
dividuals involved and is reflected in the larger standard deviations. Even
so, the relative smallness of the standard deviations and the uniformity of the
results obtained indicate that such division of the data is permissible.
Sciatic stimulation does not alter the frequency.

Table 4 contains data presented in Fig. 4, demonstrating the relationship

Table 3. *Mean frequency per second of cortical waves before and after sciatic stimulation.*
(With Standard Deviations)

Agent	Waves	Anesth. Level	Frequency Before Stim.	Frequency After Stim.
Nitrous oxide	All	Light	43.5 ±0.9	42.9 ±1.0
Cyclopropane	All	Light	43.7 ±1.0	42.8 ±0.7
	All	Deep	42.1 ±1.1	42.1 ±1.1
Ether	All	Light	39.9 ±0.5	40.2 ±0.8
	All	Deep	38.0 ±0.5	39.7 ±0.6
Divinyl ether	All	Light	38.7 ±0.8	37.5 ±0.5
	All	Deep	35.8 ±1.3	35.0 ±0.9
Ethylene	All	Light	36.9 ±0.6	36.5 ±0.5
	All	Deep	37.6 ±0.8	37.5 ±0.4
Trichlorethylene	All	Light	32.8 ±0.8	32.9 ±0.7
	All	Deep	32.8 ±0.6	32.4 ±0.7
Ethyl alcohol	All	Light	32.1 ±0.9	31.0 ±0.8
	All	Deep	30.0 ±0.5	30.9 ±0.6
Ethyl chloride	All	Light	30.4 ±1.1	27.5 ±0.7
	All	Deep	29.9 ±0.6	28.4 ±0.9
Ethyl urethane	All	Light	29.9 ±0.5	29.8 ±0.9
	All	Deep	29.7 ±0.6	29.3 ±0.7
Chloroform	All	Light	28.8 ±0.4	29.0 ±0.6
	All	Deep	26.9 ±0.8	26.4 ±0.5
Amylene hydrate	All	Light	26.3 ±1.5	25.3 ±1.5
	All	Deep	24.9 ±0.7	25.3 ±0.6
Tribromethanol	Small	Light	22.7 ±0.7	21.0 ±1.6
	Small	Deep	21.7 ±0.4	24.1 ±0.8
	All	Light	27.3 ±0.8	27.0 ±1.3
	All	Deep	25.9 ±1.9	26.8 ±1.1
"Evipal"	Small	Light	20.2 ±1.0	24.3 ±0.9
	Small	Deep	18.7 ±1.4	18.5 ±3.2
	All	Light	23.2 ±1.0	24.3 ±0.9
	All	Deep	20.6 ±0.8	19.3 ±2.9
Paraldehyde	Small	Light	20.1 ±0.5	19.1 ±0.7
	Small	Deep	21.4 ±0.9	22.2 ±1.0
	All	Light	25.3 ±0.7	23.1 ±0.5
	All	Deep	22.8 ±1.2	22.2 ±1.0
Sodium barbital	Small	Light	19.4 ±1.5	19.0 ±0.7
	Small	Deep	17.8 ±0.6	16.2 ±1.6
	All	Light	23.6 ±1.1	22.2 ±1.5
	All	Deep	21.4 ±1.0	22.0 ±1.3
Chloralosane	Small	Light	13.7 ±0.4	13.1 ±0.6
	Small	Deep	13.5 ±0.5	12.8 ±0.7
	All	Light	15.0 ±0.4	13.8 ±0.6
	All	Deep	14.2 ±0.7	13.6 ±0.3
"Nembutal"	Small	Light	13.6 ±0.5	13.1 ±0.8
	Small	Deep	16.0 ±0.6	13.3 ±1.7
	All	Light	14.8 ±0.5	14.9 ±1.0
	All	Deep	17.8 ±1.4	16.4 ±2.2

of voltage of cortical waves to depth of anesthesia. As already described, the depth of anesthesia was arbitrarily chosen on the basis of the response of lower leg flexion to central sciatic stimulation. The excursion made by the recording lever on a smoked drum was measured in millimeters. The voltage of the cortical waves just preceding the stimulation was measured in microvolts as described above (in obtaining data for Table 2). Though data on only one experiment under each agent are presented, numerous others were measured: 3 experiments for ether, 7 for cyclopropane, 2 for ethylene and so on. When the voltages are to be compared over even a short period of time
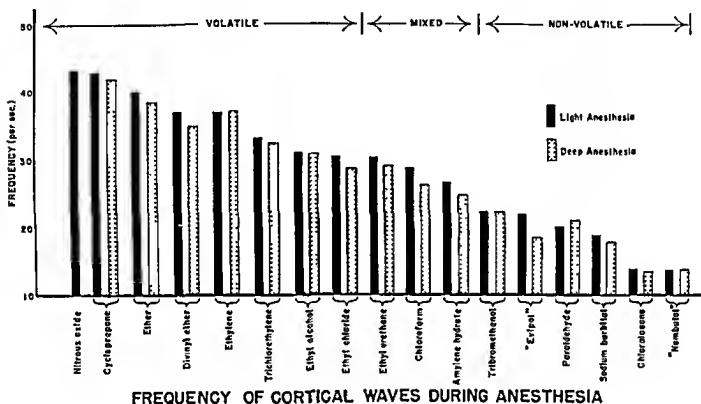
FIG. 2. This figure is based upon the fine waves. It seemed better to compare like waves. In any case, a similar figure prepared from the total wave count data would be little different from this. See Table 1.

it is necessary that the following several factors be kept constant: Amplification of the voltages, the galvanometer string tension (corrected on the basis of frequent calibrations), the strength and duration of the stimulus producing the reflex, the reflex recording apparatus, and the general condition of the animal, with particular attention to adequate oxygenation.

Pattern. While it is impossible to choose a strip of film only a few centimeters long which will demonstrate all characteristics of the action potentials for a given agent, in Fig. 1 sample records are presented and, though they are considerably reduced in size, it is evident they can be divided into two distinct classes: First, there is the group composed of nitrous oxide, cyclopropane, ether, divinyl ether, ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, chloroform, and amylene hydrate. These, with the exception of ethyl urethane are volatile agents, and the frequency
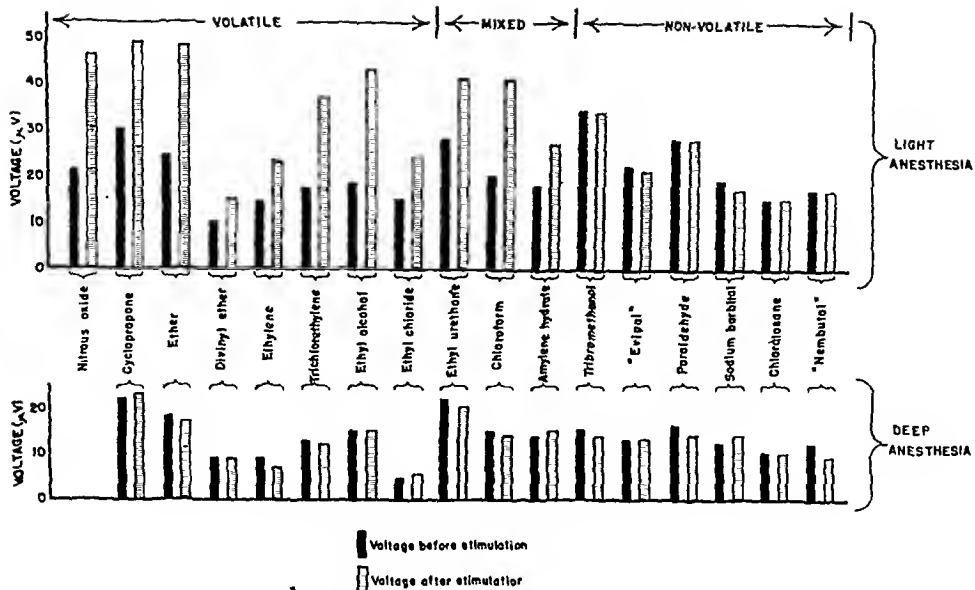
EFFECTS OF SCIATIC STIMULATION ON THE VOLTAGE OF CORTICAL WAVES DURING ANESTH.

FIG. 3. Data do not include large waves. See Table 2.
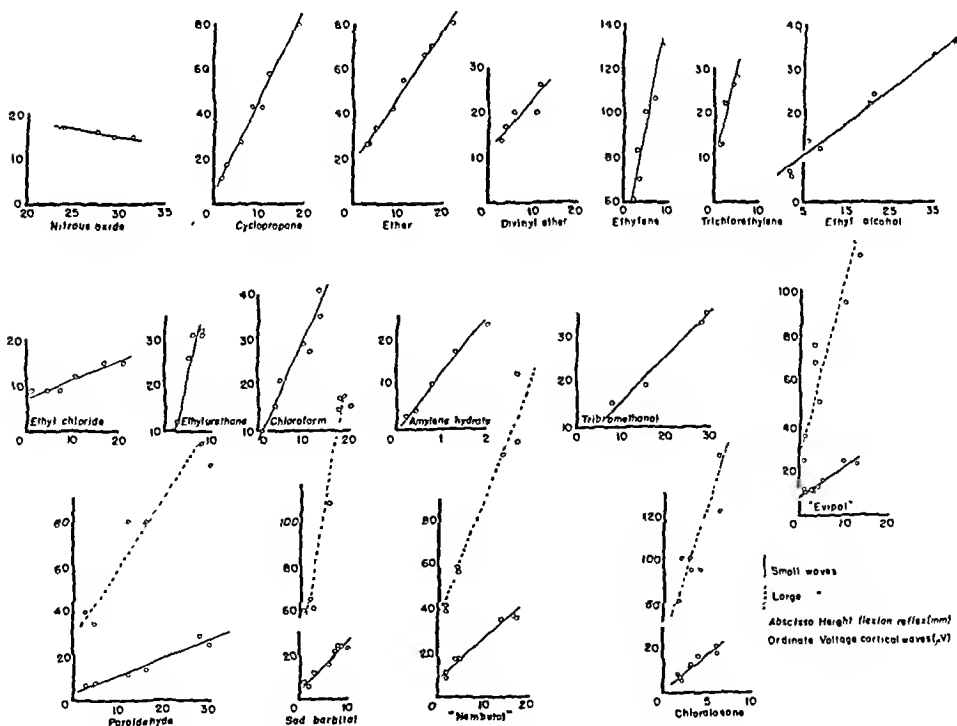
Relation of Voltage of Cortical Waves to Depth of Anesthesia

FIG. 4. See Table 4.

Table 4 Relation of the voltage of cortical waves to the depth of anesthesia
(As measured by the height of a flexion reflex)

Date	Agent	Film No	Time	Voltage of Cortical Waves* (μV)	Height of Flexion Reflex (mm)
3/25/38	Nitrous oxide	100	2 51	15	29 5
		101	2 53	15	31 5
		102	3 03	17	24 0
		109	3 09	16	27 7
3/25/38	Cyclopropane	58	12 34	12	2 0
		59	12 35	18	3 0
		60	12 36	28	6 0
		61	12 37	43	8 5
		62	12 38	43	11 0
		63	12 39	58	12 3
		65	12 41	80	18 5
		66	12 42	100	21 5
3/25/38	Ether	13	11 38	27	3 0
		14	11 39	27	3 5
		15	11 40	34	5 0
		17	11 43	42	8 7
		19	11 46	55	11 2
		22	11 49	66	15 9
		24	11 53	70	17 2
		26	11 57	80	22 0
3/25/38	Divinyl ether	110	3 22	14	3 0
		111	3 23	17	3 8
		112	3 23½	20	5 8
		113	3 24	20	10 7
		114	3 25	26	11 8
3/28/38	Ethylene	12	12 19	61	2 2
		14	12 22	70	3 9
		16	12 24	83	3 2
		18	12 27	100	4 7
		19	12 30	106	7 0
		20	12 31	130	8 5
12/29/37	Trichlorethylene	23	3 39	13	1 5
		25	3 44	13	1 8
		26	3 46	22	2 5
		27	3 55	26	4 5
		28	3 59	28	5 3
4/28/38	Ethyl alcohol	8	12 01	33	35 0
		10	12 05	36	40 0
		27	12 31	24	21 0
		29	12 33	22	20 0
		32	12 42	12	8 5
		36	12 49	14	6 0
		42	1 04	6	2 5
		45	1 09	7	2 0

Table 4 (Cont.). Relation of the voltage of cortical waves to the depth of anesthesia.
(As measured by the height of a flexion reflex)

Date	Agent	Film No.	Time	Voltage of Cortical Waves* (μV)		Height of Flexion Reflex (mm.)
7/18/38	Ethyl chloride	13	10:44	15		17.0
		14	10:45	15		21.0
		16	10:50	12		10.8
		18	10:51	9		7.2
		23	11:00	9		4.7
		25	11:02	9		1.0
5/ 2/38	Ethyl urethane	4	10:54	26		5.0
		14	11:14	31		6.0
		15	11:24	31		8.0
		16	11:34	32		8 0
		22	11:54	12		2.5
		28	12:07	10		2.5
1/ 5/38	Chloroform	5	12:23	10		1.0
		7	12:32	15		4.0
		8	12:35	21		5.0
		10	12:38	29		10.0
		11	12:40	27		11.0
		12	12:42	35		13.0
		13	12:44	41		13.0
5/ 9/38	Amylene hydrate	9	11:13	23		2.0
		11	11:18	17		1.3
		14	11:20	10		0.8
		17	11:23	4		0.4
		19	11:25	3		0.2
				Small Waves	Large Waves	
5/13/38	Tribromethanol	2	1:12	15	200	8.0
		3	1:13	19	240	15.5
		4	1:14	35	260	29.0
		5	1:16	33	220	27.5

per sec. under light anesthesia is in every case above 26. The group is characterized by rapid, fine waves. Then there is the other group composed of tribromethanol, "evipal," paraldehyde, sodium barbital, chloralosane, and "nembutal." Here the agents are all "non-volatile,"[†] having slow total frequencies. The pattern is characterized by large slow waves (1 to 10 per sec.) on which small waves are superimposed. Though the figure does not show it well, these large waves tend to come in bursts* every 3 to 5 sec., and each burst lasts 1 or 2 sec. Between the bursts the galvanometer is comparatively quiet. The characteristic patterns for the two groups remain consistent and distinct throughout all levels of anesthesia, as long as activity can be re-

† That is, can not be administered by inhalation.
* Except in the case of chloralosane.

Table 4 (Cont). Relation of the voltage of cortical waves to the depth of anesthesia.
(As measured by the height of a flexion reflex)

Date	Agent	Film No	Time	Voltage of Cortical Waves* (μV)			Height of Flexion Reflex (mm)
				Small	Large	Bursts	
4/ 7/38	"Evipal"	2	11 00	23	116	102	13 0
		5	11 15	24	95	125	10 0
		11	11 47	16	—	128	5 0
		12	12 02	13	50		4 0
		18	12 40	11	76		3 0
		20	12 46	11	68		3 0
		22	12 56	12	36		1 0
		24	1 03	11	25		1 0
7/28/38 A M	Paraldehyde	12	11 55	29	115	129	28 0
		14	11 56	25	105	136	30 0
		19	12 24	17	80	126	16 0
		20	12 34	12	80		12 0
		21	12 47	8	35		5 0
		22	12 51	7	40		3 0
4/11/38	Sodium barbital	11	2 50	23	150	280	10 0
		13	2 55	24	154	356	8 0
		14	2 58	24	155	328	8 8
		16	3 05	22	149	363	7 3
		19	3 14	16	108	340	5 5
		21	3 26	12	61	261	2 5
		24	3 43	6	65	279	1 5
		26	3 46	8	60	360	0 5
7/ 8/38	Chloralosane	6	2 32	21	122		6 0
		8	2 43	18	147		6 0
		14	3 09	13	96		3 0
		15	3 14	17	96		4 0
		16	3 19	12	101		3 0
		21	3 36	6	101		2 0
		22	3 39	7	—		1 8
		24	3 44	9	82		1 5
2/ 7/38	"Nembutal"	9	11 54	35	109	145	14 0
		12	12 01	36	115	—	17 0
		24	12 13	36	144	168	17 0
		48	2 14	17	58	105	4 0
		56	2 45	17	56	103	4 0
		66	3 16	11	41	83	1 5
		67	3 19	9	39	79	1 5

* Average for all waves unless otherwise specified.

corded. The consistency to type of anesthetic agent persists regardless of how often a given animal may be shifted from one agent to another. *Consistency of pattern is a fundamental characteristic of the behavior of the electrical activity in the cortex under a given anesthetic agent.* Detailed frequencies and voltages for each agent are given in Tables 1 and 2.

Secondary cortical discharges (see Forbes and Morison, 1939) in response to central sciatic stimulation have been found under amylene hydrate, tribromethanol, "evipal," paraldehyde, sodium barbital, chloralosane, and "nembutal." The first two compounds listed are the components of "avertin"; Derbyshire, Rempel, Forbes and Lambert (1936) reported the presence of the phenomenon under this agent, as they also did under "nembutal." The secondary cortical discharges were not found under the other agents.

Discussion

Frequency. A number of points emerge from the foregoing mass of data. The chief ones will be considered forthwith: Each anesthetic agent (see Table 1) conditions a fundamental frequency of cortical waves; determined under given conditions (animal, position and type of electrodes) this is a constant within fairly narrow limits. The frequency remains essentially unaffected throughout a wide range of anesthesia depth. In one or two cases there is a slight tendency to slowing with increase in depth of anesthesia. This may conceivably be due to the dropping out of waves of muscular origin although the arrangement of the electrodes is such as to render this improbable. In any case the change is so slight as to be of questionable significance.

It is not possible to say on the basis of the present data that there is a frequency which is only characteristic of a given anesthetic agent. For example, the difference between the mean frequency per second for nitrous oxide, 43.2 ± 0.7 is certainly not significantly different from that for cyclopropane, 42.9 ± 0.6; yet there is unquestionably a significant difference between that for nitrous oxide and ether. In this case the difference of the means is *four* times the square root of the sum of the squares of the standard deviations. In other words, it is twice as much as it would need to be for bare significance. Frequently the difference between adjacent agents in Table 1 is not significant; whereas in agents once removed the frequency usually is significantly different.

Table 3 shows that neither under light nor under deep anesthesia can sciatic stimulation affect the frequency under any of the agents studied. We can be certain that this lack of effect was not due to failure of the stimulus to reach the cortex, for in Table 2 and Fig. 3 it is apparent that the stimuli employed did reach the cortex and altered the voltages of the waves in the "volatile" and "mixed" groups while the secondary discharges associated with all of the agents in the "non-volatile" groups indicate that here also pathways to the cortex were open.

With anesthesia deeper than that employed in this study, the voltage of the waves is so reduced it becomes difficult and finally impossible to distinguish the waves; so for deep levels of anesthesia, as far as we can detect with present apparatus, the frequency does finally slow down: the waves disappear before death. *But the fact remains that for a given agent, within the range of anesthesia depth employed clinically the frequency is remarkably con-*

stant, being relatively characteristic for a given agent and little changed by alterations of depth over a wide range and unaffected by peripheral stimuli (sciatic).

When the frequencies are tabulated in order, as they are in Table 1 and Fig. 2, a further interesting point becomes evident; *viz.*, the agents with the highest cortical frequencies are highly volatile. As the frequency decreases, in general so also does the volatility, so that the non-volatile anesthetic agents appear at the opposite end of the list.

It is interesting to observe in the case of the non-volatile agents that the frequency of the cortical beat approaches more nearly to that found in un-anesthetized animals. Derbyshire *et al.* (1936) found in unanesthetized, dozing cats that the cortical potentials showed a dominant pattern of about 6 waves per sec. with a voltage of about 60 μV. Superimposed upon these waves there were smaller excursions at about 14 per sec. total of 20. We have found under light "evipal" anesthesia a total frequency of 22 with a voltage of the large waves of 86.

In addition to these similarities in frequency, similarities in pattern have been observed. Derbyshire *et al.* (1936) have called attention to the great amplitude of the large slow waves under "nembutal." The appearance of these under the barbiturate, as they point out, resembles like waves which develop with the onset of natural sleep. Bremer (1937) has also insisted that barbiturate narcosis closely resembles natural sleep. Clinicians have long been aware of the similarity of barbiturate hypnosis and narcosis and natural sleep. Indeed, almost invariably patients on going under "evipal" anesthesia yawn, and every surgeon knows that if he starts his surgical procedure before this yawn occurs he is likely to disturb his patient to such an extent it will be difficult to get him satisfactorily "asleep" without using an unusually great quantity of the anesthetic. A comparison of cortical frequencies offers some objective evidence that narcosis by means of the non-volatile anesthetic agents is more nearly akin to natural sleep than that induced by the volatile agents.

Voltage. We have seen that the frequency of the cortical waves is a relatively stable characteristic of the animal under a given anesthetic agent. The voltage, however, is rather labile. Table 2 and Fig. 3 show that under light anesthesia central sciatic stimulation greatly increases (50 to 140 per cent) the voltage of the cortical waves under nitrous oxide, cyclopropane, ether, divinyl ether, ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, chloroform, and amylene hydrate. This confirms the observation of Derbyshire *et al.* (1936) for ether. From Fig. 3 it is apparent that this response to stimulation is characteristic of agents in the "volatile" and "mixed" groups. It is lost abruptly as soon as the "non-volatile" group is entered.

The table and figure also demonstrate that in no case with any agent does stimulation alter the voltages under deep anesthesia. The table also shows that voltage of the large waves which is found in the non-volatile group is

unaffected by the sciatic stimulation; yet this lack of effect on voltage is not due to failure of impulses to reach the cortex, for it is in this very group that the secondary discharges are outstandingly apparent.

Table 4 and Fig. 4 demonstrate the close relationship between voltage of cortical waves and depth of anesthesia as measured by the height of a flexion reflex following sciatic stimulation. Except in the case of the occasional large waves there is a striking linear relation shown on graphing the data. This relationship is extraordinarily close considering the possibilities of error in measurement. Even with the occasional large waves the linear nature of the curve is clearly apparent. Waves appearing in bursts do not seem to be as uniformly depressed.

The large quantity and variety of data presented *show a clear correlation between the response of the sensory cortex and the discharge of the spinal motor neurones. The cortical voltages are not simply unrelated phenomena arising from isolated centers.*

The voltage of cortical waves under anesthesia, then, is a labile characteristic easily affected in the case of some agents by peripheral stimuli, but not affected in others, a characteristic uniformily altered by changes in depth of anesthesia.

General. There is a striking tendency for the anesthetic agents to fall into the same two groups when tested by various criteria. Any far-reaching speculation on such a basis is unwarranted, of course; but it is interesting to call attention to the common qualities of the agents of each group. A consideration of the common characteristics of the members of each group is not intended to imply that there are not many differences between the individual members. Rioch and Rosenblueth (1935) for example, have pointed out differences in the action of several non-volatile anesthetic agents.

Secondary cortical discharges (for a discussion of this phenomenon, see Forbes and Morison, 1939) in response to sciatic stimulation are *not* found under nitrous oxide, cyclopropane, ether, divinyl ether,* ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, or chloroform anesthesia.

Secondary discharges *do* appear in response to sciatic stimulation under amylene hydrate, tribromethanol, "evipal," paraldehyde, sodium barbital, chloralosane, and "nembutal" anesthesia.

It is interesting to observe that no secondary discharges were elicited under any agent having a fundamental frequency of cortical waves (total) above 28 per sec., while the secondary discharge could be elicited regularly in all of the agents having a frequency below 28. In the former group, the agents are predominantly volatile, whereas, in the latter they are mainly non-volatile.

Though the separation of the agents into similar distinct groups on the basis of alteration of a flexion reflex is not so clear cut, it is interesting to

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* On one or two occasions waves suggestive of a secondary discharge appeared in response to stimulation under divinyl ether anesthesia. These were never definite.

recall in passing (as previously reported by Beecher, McDonough and Forbes, 1939) that a sustained, *cumulative* reflex contraction of the leg flexors is regularly found upon repeated central stimulation of the sciatic nerve under one group of anesthetic agents: Nitrous oxide, cyclopropane, ether, ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, chloroform and amylene hydrate. In the earlier paper it was pointed out that the reflex under these agents is of the same type as it is in the decerebrate animal. On the other hand it was found (*loc. cit.*) under another group of agents: "evipal," sodium barbital, "nembutal" and chloralosane, that the flexion reflex response was like that which appears following sciatic stimulation in an animal after low transection of the spinal cord. The reflex here was characterized by large, isolated, *non-cumulative* twitches in response to central sciatic stimulation. The remainder of the agents did not fall as clearly into the two groups; with divinyl ether, tribromethanol and paraldehyde sometimes one type of flexion response occurred and sometimes another. The type of response elicited seems to depend upon depth of anesthesia.

The outstanding difference between the two distinct types of flexion reflex appears to be a matter of after-discharge. This is in harmony with the conclusions of Bremer and Moldaver from studies of a frog's spinal reflex (1934). With the first group, the ether-like group, the after-discharge following stimulation is little if at all reduced and the flexion response rises in a cumulative fashion as a result of the prolonged stimulation. With the second group, the barbiturate-like group, the after-discharge is greatly reduced, and the increased response to each stimulus appears as an isolated twitch of the muscle.

If the widely accepted view is correct that after-discharge is due to interruncial neurones which, through "long-circuiting" of sensory impulses provide for a prolonged stimulation of the ventral horn cells after the original excitation has ceased, then the latter group is distinguished by curtailment of the long-circuiting of sensory impulses.

When the agents are divided on the basis of the presence or absence of a secondary discharge following stimulation, the resulting two groups are rather strikingly similar to the two groups which can be made by dividing the anesthetics on the basis of type of reflex response to stimulation. There are two discrepancies here but one cannot help wondering if they may not be more apparent than real, due to experimental inadequacy rather than to fundamental difference. For example, divinyl ether at certain levels of anesthesia gives the non-cumulative type of flexion response and it has been pointed out earlier that under this agent waves suggestive of the secondary discharge appeared in response to sciatic stimulation. These were not clear-cut, and we have placed this agent in the group not showing such phenomena, since we failed to elicit an unmistakable secondary discharge. Perhaps this is an error. The other discrepancy concerns amylene hydrate. Here, clear-cut secondary discharges were produced, but not the non-cumulative

type of flexion reflex. But as already pointed out (Fig. 2) amylene hydrate is a border-line agent, falling adjacent to tribromethanol when the agents are grouped on a basis of descending frequency of cortical waves.

With these two apparent or real discrepancies present, it cannot be said that invariably with the agents under which the secondary discharges can be elicited one also gets a non-cumulative type of flexion response to stimulation, but it can be said that this is almost always the case.

When the agents are divided on the basis of whether or not sciatic stimulation affects the voltage of cortical waves, again groups are obtained which

Table 5. The agents are arranged on the basis of descending frequency of cortical waves. "1" signifies characteristic of Group I and "2" of Group II.

Agent	Molecular Size	Volatility	Frequency	Voltage	Pattern	Response to Sciatic Stimulation	Secondary Discharge	Flexion Reflex
Nitrous oxide	1	1	1	1	1	1	1	1
Cyclopropane	1	1	1	1	1	1	1	1
Ether	1	1	1	1	1	1	1	1
Divinyl ether	1	1	1	1	1	1	1 (?)	1 and 2*
Ethylene	1	1	1	1	1	1	1	1
Trichlorcthylene	1	1	1	1	1	1	1	1
Ethyl alcohol	1	1	1	1	1	1	1	1
Ethyl chloride	1	1	1	1	1	1	1	1
Ethyl urethane	1	2	1	1	1	1	1	1
Chloroform	1	1	1	1	1	1	1	1
Amylene hydrate	1	1	1	1	1	1	2	1
Tribromethanol	2	2	2	2	2	2	2	1 and 2*
"Evipal"	2	2	2	2	2	2	2	2
Paraldehyde	2	2	2	2	2	2	2	1 and 2*
Sod. barbital	2	2	2	2	2	2	2	2
Chloralosane	2	2	2	2	2	2	2	2
"Nembutal"	2	2	2	2	2	2	2	2

* The type of flexion response varies, apparently with depth of anethesia.

are strikingly similar to the preceding ones. The same thing is also generally true when the agents are divided on the basis of volatility, relatively light or heavy molecules, voltage, frequency and similar pattern. The two similar groups which appear when the agents studied are tested by 8 diverse criteria in Table 5.

As far as the criteria considered here are concerned, and notwithstanding certain discrepancies,† the rather strikingly uniform character of the two groups leads, then, to the question of whether the eight similar characteristics of each group are in any case more than chance. Typical characteristics of the *first group:* High volatility; low molecular weight; low voltage of all waves; high frequency of cortical waves; similar pattern; no secondary cortical discharge following sciatic stimulation; cumulative flexion reflex,

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† Table 5 is useful in emphasizing the discrepancies.

perhaps meaning normal long-circuiting of sensory impulses; under light anesthesia sciatic stimulation alters the voltage of cortical waves, but not under deep. For the *second group*: Low volatility; high molecular weight; high voltage of many waves; low frequency of cortical waves; similar pattern; a secondary cortical discharge following sciatic stimulation; non-cumulative flexion reflex, perhaps meaning greatly reduced long-circuiting of sensory impulses; even under light anesthesia sciatic stimulation does not affect the voltage of cortical waves here.

Any attempt to identify a common factor in the 8 characteristics of each group must be speculative. It is interesting to consider what significance, if any, there may be in the correlation between the greater reverberation and after discharge in Group I than in Group II, the increased voltage following sciatic stimulation in light anesthesia in Group I (absent in Group II), and the higher frequency of cortical waves in Group I than in Group II. The reverberation hypothesis easily accounts for the difference in type of spinal reflex observed, if one holds the widely accepted view that after-discharge is the result of continued stimulation of the ventral horn cells brought about by the long-circuiting of impulses through internuncial neurones after the original excitation has ceased, for the outstanding difference between the two types of reflex response seems to be a matter of after-discharge. The basic difference between the action of the two groups *may* be a matter of the possibilities for long-circuiting or reverberation of impulses in the central nervous system. Such a hypothesis is mainly speculative with only a little experimental evidence to support it.

Gerard and his coworkers suggested (1936) that anesthesia may "suspend awareness by lessening cellular activity or disrupting the whole active pattern." Perhaps the foregoing material points the way, at least to some extent, to how this occurs. The principal findings which have been enumerated and discussed are strikingly consistent; but interpretation of them is difficult, if not impossible as yet. These findings, we believe, must be reckoned with in any final interpretation of the electrical activity of the cortex.

SUMMARY

1. Seventeen anesthetic agents, nitrous oxide, cyclopropane, ether, divinyl ether, ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, chloroform, amylene hydrate, tribromethanol, "evipal," paraldehyde, sodium barbital, chloralosane, and "nembutal" have been studied in the cat. Following exposure of the right posterior sigmoid gyrus of the cortex, recording electrodes of the concentric type were placed on the sensory area, in all cases. Factors compared were pattern, frequency, and voltage of the cortical action potentials, as well as the response of these factors to sciatic stimulation at the several levels of anesthesia. Simultaneous spinal activity was recorded by means of the flexion reflex. Two levels of anesthesia were considered in particular: (a) light, the lightest anesthesia it was possible to work with without producing a disturbing generalized muscular re-

sponse on stimulating the sciatic nerve; (b) deep, the level at which the flexion reflex just disappeared.

2. The frequency per second for each agent at both light and deep levels of anesthesia has been determined and the mean with its standard deviation listed in all cases (Table 1, Fig. 2). For a given agent within the range of anesthesia depth employed clinically, the frequency is remarkably constant, being relatively characteristic for a given agent and little changed by alterations of depth over a wide range of anesthesia (Table 1) and unaffected by peripheral stimuli (sciatic), Table 3. When the frequencies are tabulated in order of size it is apparent that, in general, high frequencies are associated with highly volatile agents and low frequencies with non-volatile agents. Attention is called to the fact that the pattern and frequencies associated with the non-volatile agents are similar to those found during natural sleep. The generalization can be made that frequency of the total cortical waves is a relatively stable characteristic during anesthesia.

3. During *light* anesthesia under "Group I" agents (nitrous oxide, cyclopropane, ether, divinyl ether, ethylene, trichlorethylene, ethyl alcohol, ethyl chloride, ethyl urethane, chloroform and amylene hydrate), sciatic stimulation greatly increases the voltage of the cortical waves (Table 2, Fig. 3). It is also apparent there that even under light anesthesia, at least of the level used in this study, central sciatic stimulation has no effect on the voltage of the cortical waves under the "Group II" agents (tribromethanol, "evipal," paraldehyde, sodium barbital, chloralosane, and "nembutal"). Group I consists of volatile agents (excepting the urethane), and Group II of non-volatile.

Stimulation has no effect on the voltage of the typical cortical waves under any agent at a deep level of anesthesia. Attention is called to the fact that failure of stimulation to alter the voltages of any of the waves under the non-volatile agents is not due to failure of impulses to reach the cortex for "secondary discharges" occur here in response to stimulation even (and usually) at deep levels of anesthesia. This indicates that pathways remain open. There is a close linear relationship between voltage of cortical waves and depth of anesthesia as measured by the magnitude of the flexion reflex following central sciatic stimulation (Table 4, Fig. 4). The cortical voltages are a fundamental general characteristic of the cortex and not simply phenomena arising from isolated centers. The voltage of cortical waves under anesthesia is a labile characteristic easily affected in certain given cases by peripheral stimuli, but not affected in others, a characteristic uniformly altered by changes in depth of anesthesia.

4. Records showing typical action potentials for each agent at both light and deep levels of anesthesia are shown, Fig. 1. Here again, it is apparent on the basis of pattern, that the anesthetics studied fall into the two groups. The cortical waves are fine under Group I agents, fairly uniform in size, and of a frequency per second greater than 26 in all cases. In *Group II* small fine waves are superimposed on large slow waves. The large waves

tend to come in bursts (except chloralosane). Consistency of pattern is a fundamental characteristic of the behavior of the electrical activity in the cortex during anesthesia.

5. The seventeen agents studied show a tendency to fall into two groups when tested by several criteria, Table 5. The composition of the groups is strikingly similar whenever any of the characteristics is considered; viz., molecular size, volatility, frequency per second of the cortical waves (fast or slow), voltage, pattern, presence or absence of secondary discharge following sciatic stimulation, type of flexor response to sciatic stimulation, ability of sciatic stimulation to alter the voltage of cortical waves under light anesthesia.

6. Possible implications and relationships of the above findings to each other are discussed.

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THE CEREBRAL ACOUSTIC AREA OF THE CAT

A COMBINED OSCILLOGRAPHIC AND CYTOARCHITECTONIC STUDY

F. BREMER AND R. S. DOW*

Laboratoire de Pathologie Générale de l'Université Libre de Bruxelles

INTRODUCTION

THE EXACT limits of the cortical acoustic area in mammals are not yet known. Previous workers, using anatomical, physiological or pathological methods have been unable to establish the boundaries with certainty and many conclusions have been contradictory, as the following historical note indicates.

HISTORICAL NOTE

One of the earliest anatomical observations pointing to the location of the cat's acoustic area was that of Vogt (1898), who discovered a separate area of early myelinization in the region of the middle ectosylvian gyrus. Cytoarchitectonic studies of the temporal cortex of the cat have been made by Campbell (1905), Winkler and Potter (1914) and Kornmüller (1933–1937), but there is no agreement among these investigators. Campbell divided the temporal cortex of the cat into two parts, the ectosylvian A and the ectosylvian B (Fig. 1a). He regarded the former as the primary acoustic area which received the projection from the medial geniculate body. Winkler and Potter attempted to apply Brodmann's (1909) classification of the temporal lobe to their study of the cat, but their division is difficult to follow and no map was prepared. Kornmüller has described in this region a sensory type of cortex (Fig. 1b) principally characterized by a well developed fourth layer and the arrangement of the cellular constituents of this granular layer in conspicuous radially oriented rows. As will be seen below, he correlated this cytoarchitectonic appearance with the area giving electrical potentials in response to sound. The cytoarchitectonic areas of the temporal region in other laboratory mammals have been studied by Droogleever Fortuyn (1914) in 9 rodents, Tsuneda (1937) in the mouse, and in monkeys by Walker (1937) and von Bonin (1938). All have found a localized area of sensory type of cortex which they have thought to be the acoustic projection area. This is probably homologous with the areas 41 and 42 of Brodmann's parcellation, which are located on the transverse temporal gyrus of Heschl in man.

Experimental anatomical methods have been applied to this problem by various workers, likewise without total agreement (Yoshido, 1924; Ohnishi, 1931, Posthumus Meyjes, 1934; and D'Hollander and Stoffles, 1937 on rabbits; Waller, 1934, and Pennington, 1937, rats; Poljak, 1932, Le Gros Clark 1936, and Walker 1937, and Rundles and Papez, 1938, monkeys). The majority is agreed that there is a point-to-point relationship between specific parts of the medial geniculate body and specific regions of the auditory projection area. According to Walker (1937), this area as determined by retrograde degeneration of the cells in the medial geniculate body after cortical ablation, corresponded to the area of "koniocortex" which he described on the surface of the temporal lobe lying within the sylvian fissure. The agreement, between the cytoarchitectonic sensory area and the projection area from the medial geniculate body as determined by the investigators mentioned above, is apparently not as close in rodents as in monkeys.

Poljak (1927) and Mettler (1932) studied the degeneration resulting from lesions in the temporal cortex of the cat. Mettler gave particular attention to the association fibers from this region, and concluded that, instead of spreading out in a centrifugal spray arrangement, there was a tendency for the fibers to be distributed in accordance with the

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cytoarchitectonic areas as outlined by Campbell Lesions indicated that ectosylvian area A (Fig 1a) sends short association fibers in greatest number first to the rest of this area and next to the middle part of the ectosylvian gyrus Large lesions of ectosylvian area A caused association fibers to the anterior and posterior parts of the ectosylvian gyrus and to the middle suprasylvian gyrus to degenerate in considerable numbers An animal sacrificed 30 days after ablation of the ectosylvian area A had a retrograde degeneration to the homolateral medial geniculate body It is not clear from this work whether all the auditory
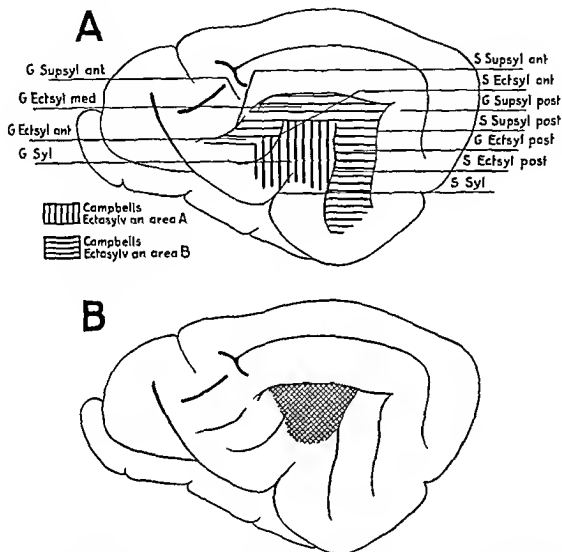
FIG 1 A Diagram of the lateral surface of the cat's cerebrum showing the sulci and gyri of the acoustic and surrounding areas The cytoarchitectonic areas according to Campbell are given

B Cortical acoustic area in the cat according to Kornmuller G Ectsyl ant , Anterior ectosylvian gyrus, G Ectsyl med Middle ectosylvian gyrus, G Ectsyl post , Posterior ectosylvian gyrus, G Supsyl ant , Anterior suprasylvian gyrus, G Supsyl post , Posterior suprasylvian gyrus, G Syl , Sylvian gyrus, S Ectsyl ant , Anterior ectosylvian sulcus, S Ectsyl post , Posterior ectosylvian sulcus, S Supsyl ant , Anterior suprasylvian sulcus, S Supsyl post , Posterior suprasylvian sulcus, S Syl , Sylvian sulcus

projection from the medial geniculate body terminates in the ectosylvian area A, or whether some may also go to the ectosylvian area B

Ades, Culler and Mettler (1938) in a preliminary report of the degeneration resulting from lesions of the medial geniculate body in the cat, describe as the largest, and apparently only cortical, connection a group of efferent fibers to the sylvian cortex A more complete report of the physiological findings in this interesting work has just appeared (Ades, Mettler and Culler, 1939) and the completion of the anatomical studies is eagerly awaited

Woollard and Harpman (1939) working independently have described the cortical projection in 2 cats with electrolytic lesions in the medial geniculate body made with a

Horsley-Clarke apparatus. Using the Marchi method, fibers have been traced to the middle ectosylvian gyrus and the posterior part of the anterior ectosylvian gyrus together with the upper part of the anterior and posterior sylvian gyri. The inferior border lies a short distance above the rhinal fissure.

Cortical ablation experiments with subsequent observation of auditory ability have been made by Munk (1890) and Larionow (1899) and others in the dog, and by Wiley (1932) and Pennington (1937), using modern conditioned reflex technique, in the rat. The latter authors review the literature concerning this type of experiment. Clinical and pathological studies in man have been made by Henschen (1918), Pfeifer (1921) and Börnstein (1932), and myelogenetical studies by Flechsig (1920). The auditory center has been described by all these as sharply localized in the region of the transverse temporal gyrus, although there is disagreement concerning the presence or absence of tonal localization within the cortex.

Physiological methods have been applied to this problem since the work of Ferrier (1876) who was the first to report an area in the temporal region of the cat and other mammals, the stimulation of which caused a movement of the contralateral ear. He concluded that this was the region of the auditory sensory area. In recent years the oscillographic technique has been applied to this problem. Gerard, Marshall and Saul (1933) briefly described electrical potentials derived from the temporal cortex in monkeys in response to sound, and in 1936 the same authors found auditory responses in the cat in the cortical grey matter, in some instances the surface of the median and posterior ectosylvian and suprasylvian gyri, and in two instances the posterior splenial gyrus. Davis (1934) determined the acoustic area in the cat by the oscillographic recording of the response to brief interrupted sounds. The boundaries which he gave in the cat extend somewhat more posteriorly than the area given by Kornmüller (Fig. 1b). Kornmüller (1933) obtained electrical potential responses to sound from a localized area in the ectosylvian region in the cat and concluded that the limits of this area as mapped out physiologically corresponded to the cytoarchitectonic area which he described. In 1937 he published a diagram of the extent of this area together with a detailed description of the different layers of the cortex in this region.

The goal of the present work is to verify and render more exact this coincidence between the cytoarchitectonic area and the area responding to sound. This verification has been made particularly desirable by the recent communication of Ades, Culler and Mettler (1938, 1939) on the functional organization of the medial geniculate bodies in the cat. It is possible that some of the discrepancies in the results may be explained by the existence of a primary sensory area and a gnostic sensory cortex, between which a distinction cannot be made by the oscillographic method as at present applied.

<div align="center">METHODS</div>

In the course of other work on the acoustic area in the cat, one of us (F. B.) has shown repeatedly, corroborating the work of Kornmüller (1933–1937), that the cortical potentials

in response to sound were best obtained from the area between and dorsal to the anterior and posterior ectosylvian sulci and ventral to the suprasylvian sulcus When the animal is not anesthetized (method of "l'encéphale isolé") and with its brain in good functional condition, the cortical response to a brief sound stimulus is made up of two successive but distinct elements (1) a large diphasic wave (Fig 2 D G) and (11) a more or less prolonged rhythmic after discharge (Fig 2 D, Bremer 1937 a and b, 1938) Of these two elements the primary wave is much more resistant, and as a consequence the more constant Further more, the after discharge has a tendency to irradiate to a distance from the source, as indicated by the primary wave (Fig 2 C) This tendency to intracortical irradiation is still more marked when the stimulus is a prolonged sound, for example, a continuous pure tone This is probably attributable to the phenomenon of summation or synaptic facilitation (Adrian, 1936) For these two reasons the observation and recording of the primary wave in response to a brief sound stimulus (click) is the best method to determine the cortical acoustic area However, the assumption that the large primary wave provoked by a brief sound stimulus characterizes oscillographically the area of projection of the geniculate cortical fibers is only an assumption Certain arguments of probability may be invoked in its behalf The demonstration, in confirmation of that of Kornmuller, of the coincidence of the cortical region thus deliminated with a well defined cytoarchitectonic area of sensory structure would lend support to this assumption

In the two experiments here reported the exact boundaries of the area giving response to "clicks" was determined by systematically moving the cotton electrodes until the borders of the responsive area were located This was done either by moving both the bipolar electrodes so that the line joining them was parallel to the expected boundaries of the region, or by placing one electrode well off the acoustic area (indifferent electrode) and moving the second in every direction, until the boundaries were located The former method gave the sharpest boundaries Throughout the experiments care was taken to maintain the distance between the electrodes as constant as possible, in order to eliminate all variation of amplitude of registered potentials which could be caused by a difference in the electrical resistance of the tissue between the two electrodes The points tested were carefully marked on a sketch of the exposed area and the presence or absence of response was recorded for each spot, as well as repeated visual observations without recording on the camera The electrocorticogram was registered with a Dubois oscillograph and a five stage amplifier coupled with condensors of 0 1μF The necessary sections of brains were fixed in 95 per cent alcohol, embedded in celloidin, sectioned serially at 25μ thickness and stained with the Nissl or with a dark Van Gieson techniques In one brain every 25th section was mounted and in the other 2 sections were mounted in every 25, one being stained with the Nissl technique and the other with a dark Van Gieson for myelin sheaths The cytoarchitectonic areas were mapped out in each case

EXPERIMENTAL RESULTS

The results in Experiment 1 are presented in tabular form in the following protocol The points explored are marked by letter on the diagram Fig. 3a and the letters correspond to the records shown in Fig. 2

Experiment 1 Cat, Jan 1, 1938 Sensitivity 10 mm per 100 μV Both bipolar and "monopolar" derivation as described above were used The indifferent electrode was placed on the posterior suprasylvian gyrus Records made using "monopolar" derivation

Point	Location	Trials recorded	Response
A	Anterior suprasylvian gyrus	1	None
B	Middle suprasylvian gyrus	2	Slight to none
C	Anterior ectosylvian gyrus	1	Slight
D	Middle ectosylvian gyrus	4	Marked
E	Posterior ectosylvian gyrus (superior part)	3	Moderate to absent
F	Posterior ectosylvian gyrus (middle part)	1	None
G	Sylvian gyrus	1	Marked
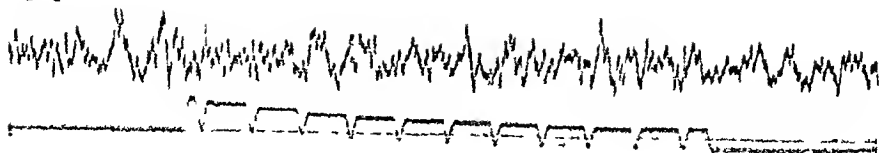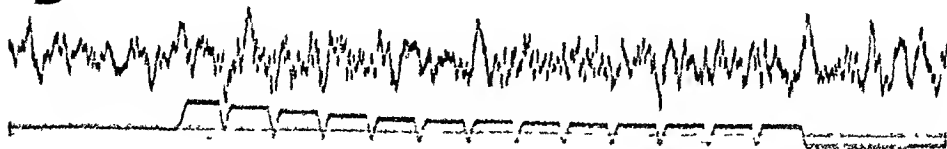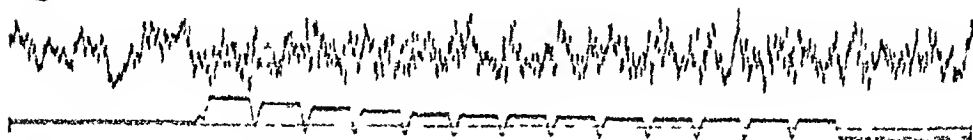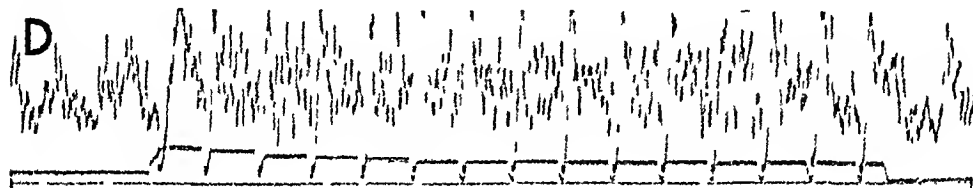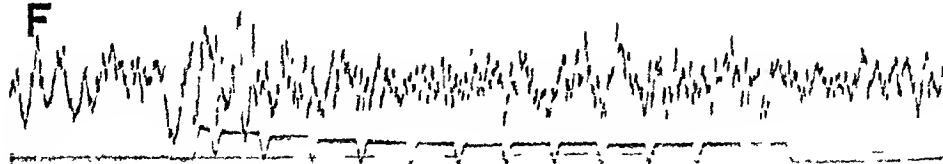
FIG. 2. The responses of the acoustic cortex to "clicks" in Exp. 1. "Monopolar" leading. Sensitivity 10 mm. per 100 μV. Original reduced $\frac{1}{2}$. The letters refer to the diagram Fig. 3a and indicate the position of the lead electrode when the record was taken. Records from above downward show: (i) electrocorticogram, (ii) signal of the occurrence of the "clicks" and (iii) time in seconds. For other explanations see text.

Figure 2, a reproduction of some of the records demonstrates the marked differences in response resulting from slight changes in the position of the active lead. Record C shows the response of the cortex at the anterior bor-
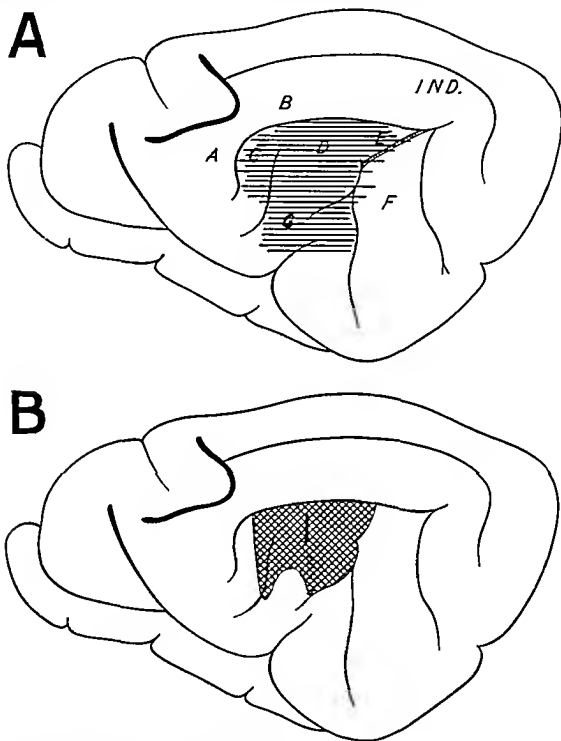
FIG. 3. Diagrams of the cortical markings in Exp. 1.
   A. The area giving responses indicated by shaded area. Letters A to G indicate the points from which the records were taken which are shown in Fig. 2.
   B. The cytoarchitectonic area as mapped out in Exp. 1. The vertical line within the area indicates the site of the microphotograph shown in Fig. 4.

der of the area (point C, Fig. 3A) with a definite acceleration of the spontaneous waves, without primary waves in response to the "clicks." This phenomenon, which is probably the expression of the intracortical irradiation of the after-discharge, is entirely absent in record A taken from a lead

on the anterior suprasylvian gyrus (point A, Fig. 3A). This point is only a few millimeters in front of point C. In the record E from the posterior border of the responsive area (point E, Fig. 3A) the primary waves are visible, but are small when compared with the same waves in records D and G taken from the center and inferior part of the area. Records B and F, taken respectively from the middle supra-sylvian gyrus and middle part of the posterior ecto-sylvian gyrus (points B and F, Fig. 3A), show an absence of response, save for a slight acceleration of the spontaneous waves of the corticogram.
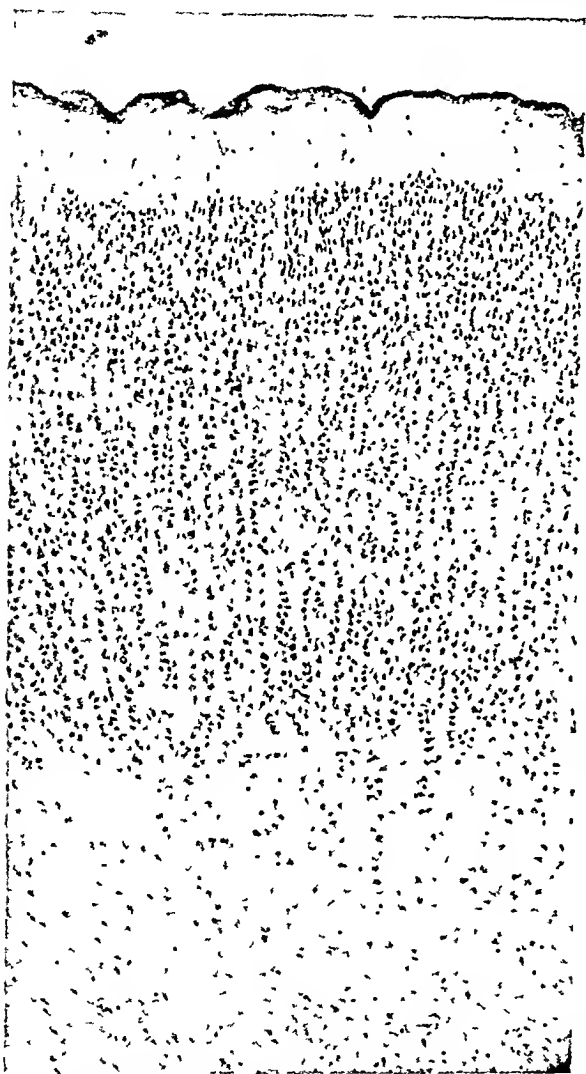
FIG. 4. Microphotograph of a portion of section taken from Exp. 1. The section is from the middle ectosylvian gyrus within the acoustic area. 50 ×.

The shaded area in Fig. 3A is the total extent of the responsive area. The cortical area having cytoarchitectonic characteristics of a "sensory" cortex makes up "b" in Fig. 3. This cortex has a broad internal granular layer, the cells of which are arranged in conspicuous radial rows and a markedly reduced internal pyramidal layer. A section from the middle ectosylvian gyrus in the center of the acoustic area is shown in Fig. 4. It is generally agreed among students of cytoarchitectonics that the boundaries of the various areas in the cat are less clearly defined than in many other mammals, including certain rodents. This is particularly true of the temporal cortex which even in primates is more difficult to divide than are other lobes with sensory areas.* (von Bonin, 1938)

* The authors are indebted to Dr. A. E. Kornmuller who kindly confirmed our identification of this area and who checked the boundaries of the area in a few of our own sections.

The results in Experiment 2 are given in the following tabular protocol. The points explored are indicated by number on both the table and the diagram, Fig. 5A. In this case both electrodes were placed on the active region being about 4 mm. apart. The position of the figures in the diagram (Fig. 5A) indicates a central point between the two electrodes. Points 1 to 7 are with the line between the two electrodes vertical, and thus parallel to the anterior and posterior borders of the active area. Points 8 to 13 are with the line between the two electrodes horizontal, and thus parallel with the superior and inferior borders of the acoustic area.

Experiment No 2 Cat, Mar 15, 1938 Sensitivity 10 mm per 100 μV. Records made with bipolar derivation Points refer to numbers on diagram, Fig. 5A

Point	Location	Trials Re-corded	Response
1	Posterior border of the posterior ectosylvian gyrus	2	None
2	Posterior ectosylvian gyrus	3	None
3	Boundary between middle ectosylvian and posterior ectosylvian	3	Marked
4	Middle ectosylvian gyrus	1	Marked
5	Middle ectosylvian gyrus	1	Marked
6	Anterior ectosylvian gyrus	1	Moderate, but irregular and without after-discharge
7	Anterior boundary of the anterior ectosylvian gyrus	1	None
8	Middle suprasylvian gyrus	2	None
9	Middle ectosylvian gyrus	5	Marked
10	Middle ectosylvian gyrus	2	Marked
11	Boundary between middle ectosylvian gyrus and sylvian gyrus	1	Marked
12	Superior border of sylvian gyrus	3	Marked
13	Sylvian gyrus	3	Moderate

The extent of the acoustic area as determined by histological methods is presented in Fig. 5B. The close correspondence between the cytoarchitectonic area and that giving potential responses is obvious in all except the inferior boundary. This may be explained by the difficulties in leading from the depths of the cortical exposure without touching more dorsal regions. In other more favorable experiments the inferior boundary has also been found to correspond to the inferior border of the sensory area, as outlined histologically in these two cases. The authors are in agreement with Kornmüller (1937) in regard to the histological and physiological boundaries of the acoustic area in the cat. We are unable to confirm the cytoarchitectonic areas as given for the cat by Campbell (1905) or Winkler and Potter (1914). The study of association fibers in this region by Mettler (1932) seemed to conform to the parcellation as given by Campbell. However, in view of the fact that the largest number of short association fibers leaving the sylvian gyrus went to the middle ectosylvian gyrus, this might be interpreted as
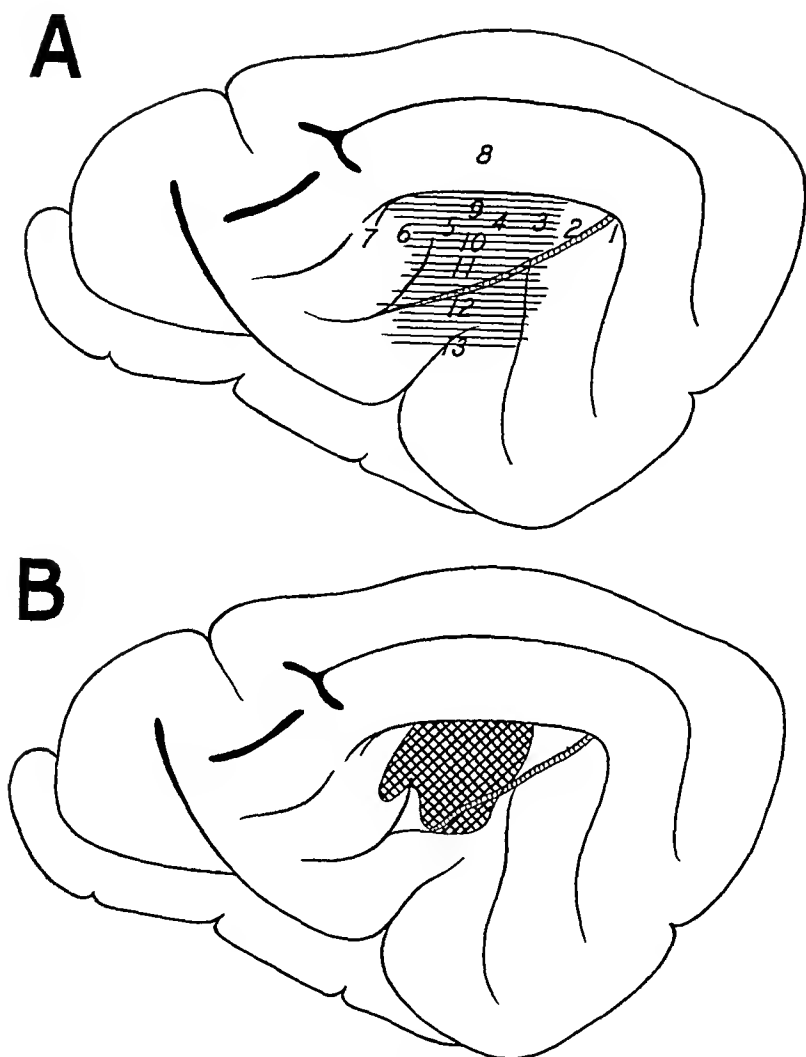
FIG. 5. Diagrams of the cortical markings in Exp. 2.

A. The area giving responses indicated by the shaded area. The numbers 1 to 13 indicate the points tested with "bipolar" electrodes and refer to the numbers in the tabular protocol of Exp. 2.

B. The cytoarchitectonic acoustic area as mapped out in Exp. 2.

supporting the present delimination. Of utmost importance will be the findings of Ades and his collaborators with the use of the Horsley-Clarke apparatus concerning the exact limits of the projection from the medial geniculate bodies after isolated lesions there. The cerebral acoustic area here described is identical with the area of projection of the fibers from the medial geniculate body as recently determined in two cats by Woollard and Harpman (1939).

In confirming the boundaries of this area we do not wish to infer that it

is necessarily strictly uniform in cytoarchitectonic structure Indeed, more rigid criteria than we have been able to apply to this limited material will possibly show the existence of subdivisions within the acoustic area thus deliminated

CONCLUSIONS

1 The boundaries of the area giving responses to auditory stimuli as determined by Kornmuller in the cat have been confirmed These boundaries correspond to definite cytoarchitectonic boundaries The whole area giving responses, although similar in structure, may not prove to be a uniform field when more rigid criteria for division are applied

2 This area includes the upper part of the sylvian gyrus, the posterior part of the anterior ectosylvian gyrus and the middle ectosylvian gyrus It is thought that this region represents the auditory projection area in the cat

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FACTORS FOR FACILITATION AND EXTINCTION IN THE CENTRAL NERVOUS SYSTEM*

J G DUSSER de BARENNE AND W S. McCULLOCH

*Laboratory of Neurophysiology, Yale University
School of Medicine, New Haven, Conn.*

CONTENTS

INTRODUCTION

THE PHENOMENON now known as "facilitation" was discovered in 1881 by Bubnoff and Heidenhain[7] who observed that, with an interval of only a few seconds, repetition of supraliminal stimulation of a "motor" focus of the cerebral cortex elicited a larger peripheral response with shorter latency, and even that repetition of subliminal stimulation could bring in a response. They also demonstrated augmentation of motor response to cortical stimulation by antecedent cutaneous stimulation of the limb in which the response occurred. These phenomena are called facilitation. Facilitation of

motor response can also be induced by antecedent stimulation of a neighbouring allied cortical focus (Graham Brown's "secondary facilitation"). What is common in all these instances is that *antecedent stimulation* has so altered the functional condition of the central nervous system (CNS) that the response to subsequent stimulation (*i.e.* to *test stimulation*) is changed in the direction of *more* response. To produce this effect the antecedent stimulation must excite, and we define here as factors for facilitation any changes of the CNS caused by antecedent excitation and causing a fall in threshold, a decrease in latency, an increase in amplitude or any combination of these.

The phenomenon designated "extinction," discovered in 1934 by the present authors, is a diminution or absence of response on repetition of stimulation of a "motor" focus within an interval longer than that required for facilitation. The latency of a partially extinguished response is markedly greater than of that to test stimulation alone. Subliminal antecedent stimulation of the same focus may yield extinction.[11,13] What is common in all these is that *antecedent stimulation* has so altered the functional condition of the CNS that the response to test stimulation is changed in the direction of *less* response. The antecedent stimulation must excite, and we would define as factors for extinction any changes of the CNS caused by antecedent excitation and causing a rise in threshold, an increase in latency, a decrease in amplitude or any combination of these.

In all that follows one must not confuse extinction—the diminution of motor response resulting from antecedent excitation of the parts of the CNS responsible for that same response—and inhibition, *i.e.* the diminution of motor response resulting from contemporaneous excitation in parts of the CNS responsible for an antagonistic response. Thus, though extinction and inhibition both result in diminution of motor response, inhibition depends upon reciprocal innervation, extinction does not.

METHODS

All experiments were performed on *Macaca mulatta* monkeys, fully anesthetized with Dial* (Ciba), 0.45 cc. per kg. bodyweight, half of the dose given intraperitoneally, half intramuscularly.

The duration of the electrical stimulations and the intervals between them were rigidly controlled by the timing-device previously described.[13] The pulses used to stimulate the cortex were either 60∿ through a center-tap transformer grounded at its midpoint† or those of a Thyratron-stimulator after Schmitt and Schmitt,[36] permitting independent variation of the number of pulses per second (pattern-frequency), and the shape, or duration, of the individual pulses (pulse-frequency) as well as the voltage. These voltages are in this paper given in terms of the readings (in Ω) on a decade voltage-divider (VD) of 10,000 Ω.

The apparatus and electrodes used for the various purposes of the particular groups of experiments will be specified later. Suffice it here to say that for simple stimulation and for recording of the ordinary electrical activity of the various portions of the CNS regular stigmatic Ag-AgCl electrodes were used. In those cases in which "after-potentials" or

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* We wish to thank the Ciba Co. for kindly putting the Dial at our disposal.

† A precaution to eliminate as much as possible polarization, kindly suggested by Dr. Hallowell Davis.

other slow components were studied special Agar-Ag-AgCl electrodes were used in combination with a D.C. amplifier and cathode ray oscillograph or with a Burr-Lane-Nims microvoltmeter.[6] The pH determinations were performed by a method previously described.[16][17] The peripheral motor responses were recorded isotonically by tambours either on smoked paper or photographically with the cathode ray oscillograms.[31] The Agar-Ag-AgCl electrodes were made for us by Dr. L. F. Nims, who also gave his invaluable coöperation in those experiments in which pH was recorded.

The designation of the various areas of the sensorimotor cortex is that given in a previous paper.[14]

RESULTS

Since both facilitation and extinction are changes in motor response, these can be observed only by comparing the response to *test* stimulation following *antecedent* stimulation with the response to test stimulation alone. This comparison presupposes that the physical properties of the test stimulus are constant, which depends upon constancy of the circuit both external to the animal and within the animal. This constancy has been established experimentally with a method previously described,[25] which permits determination of the instantaneous voltage and current through the specimen during stimulation.

A. *Necessity of Antecedent Neural Excitation*

In order to conclude that differences in response to constant test stimulation result from neural *excitation* it is necessary to exclude changes due
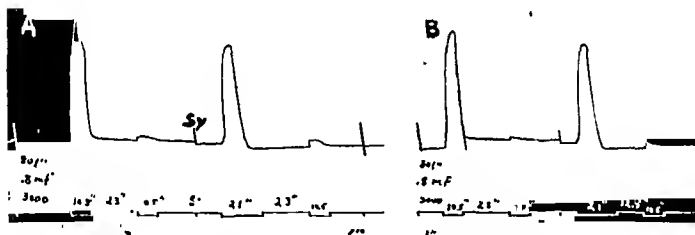
FIG. 1. Oct. 22, 1936. *Macaca mulatta*. Dial-narcosis. Record A shows 2 pairs of stimulations with all parameters the same, except that the antecedent stimulation in the 2nd pair is twice as long as that in the 1st pair. No increase in extinction. Record B repeats record A, except that the interval in the 2nd pair is half that in the 1st pair. Again extinction is not increased. Thus, whether one figures the effective interval as that between stimulations or that between responses, doubling the duration of the antecedent stimulation used here failed to increase extinction. In this and all subsequent figures, "sy" indicates synchronous points.

merely to the passage of the stimulating current. Gross changes of this nature were excluded indeed in experiments using the method above. Furthermore, the findings enumerated below are not explicable in terms of such changes: (i) motor response to cortical stimulation can be facilitated by

cutaneous stimulation (Bubnoff and Heidenhain); (ii) prolonged stimulation far enough below threshold does not produce facilitation or extinction; (iii) antecedent stimulation of such a duration that the response begins to decline before the end of stimulation produces as much extinction as does a stimulation twice as long (see Fig. 1); (iv) secondary facilitation, though present from widely separated foci in one subdivision of the sensorimotor cortex, is absent from near foci across the functional boundaries between leg-, arm- and face-subdivisions (see Fig. 2). This type of facilitation must obviously depend upon activity propagated transsynaptically, which is necessarily neural activity.
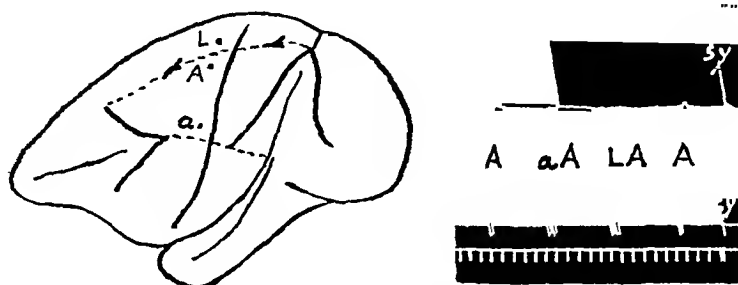
FIG. 2. Oct. 27, 1936. *Macaca mulatta*. Dial-narcosis. This figure shows the existence of secondary facilitation at A from a, 8 mm. apart, and the absence of facilitation across the functional boundary between the leg- and arm-subdivisions, although A and L are only 3 mm. apart.

From the above findings it is clear that observed differences in response, whether facilitation or extinction, cannot be referred to changes produced by *mere passage* of current of the antecedent stimulation, but must be referred to changes in the CNS resulting from neural excitation elicited by the antecedent stimulation.

B. *Multiplicity of Factors*

Some justification is required for speaking of factors for facilitation and factors for extinction instead of merely referring the two phenomena to increase and decrease of some single entity such as "excitability." Scrutiny of the following observations discloses the justification: (i) Facilitation and extinction can coexist.[9,10] If three equal stimulations are applied to one cortical "motor" focus in such temporal relation that the second and third both fall during the period of extinction induced by the first, but the third falls in the period of facilitation of the second, then the third response shows clear evidence of facilitation, though not as much as when the first stimulation is omitted (see Fig. 3); (ii) Facilitation by one criterion may concur with extinction by another. For example: a decrease in amplitude (extinction) may be associated with a decrease in latency (facilitation) (see Fig. 4); (iii) Facilitation of response to one type of test stimulation and extinc-

tion of response to another can be produced by a single type of antecedent stimulation (see Fig. 5).

It is difficult to conceive how this group of observations can be explained on the basis of changes in any *one* single parameter of nervous activity, *e.g.* in excitability. Nor is the difficulty solved, so far as we can see, by taking excitability as a variable, *i.e.* as being different at one time and place in the CNS from what it is at another. Thus we felt compelled to search for several
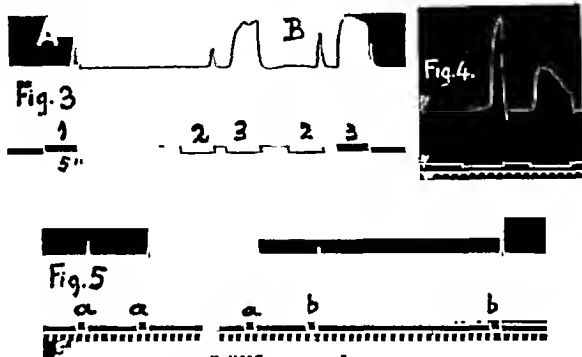
FIG. 3. Jan. 20, 1936. *Macaca mulatta.* Dial-narcosis. In record A three equal stimulations are applied to a "motor" focus. The response to stimulation 2 is extinguished, at this long interval, by the excitation set up by stimulation 1. The response to stimulation 3 is facilitated, at this short interval, by the excitation set up by stimulation 2. Record B shows the facilitation, at this interval, without extinction when stimulation 1 has been omitted.

FIG. 4. Oct. 16, 1936. The response to the second stimulation of a "motor" focus shows a shorter latency (facilitation) and a smaller amplitude (extinction) than the response to the first stimulation of this focus, *i.e.* dissociation of latency and amplitude.

FIG. 5. Jan. 31, 1935. *Macaca mulatta.* Dial-narcosis. Stimulation "a" facilitates response to "a", "a" extinguishes response to "b", as seen by comparing it with response to "b" alone.

factors for facilitation and extinction. That these factors had to be conceived as variables having different values at different times and places follows from the results of the following type of experiment, in which four stigmatic Ag-AgCl electrodes,—1, 2, 3 and 4,—are placed 1.5 mm. apart on a given "motor" area (*e.g.* Arm 4) on a straight line. Their positions have to be such that the primary movements obtained on monopolar stimulation of each of these four foci have an element in common, say extension of the wrist. It is then possible to investigate the effect of antecedent stimulation of focus 1, 2, 3 or 4 upon this common element of the response to test stimulation to focus 1 and thus to ascertain the spatial distribution of facilitation and extinction combined. By changing the interval between antecedent and

test stimulation one can thus study the intensity of facilitation and extinction as a function of space (the separation of electrodes) and time, or by
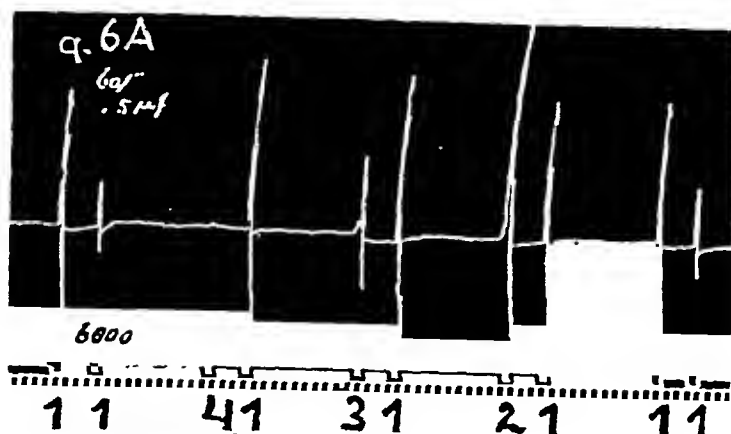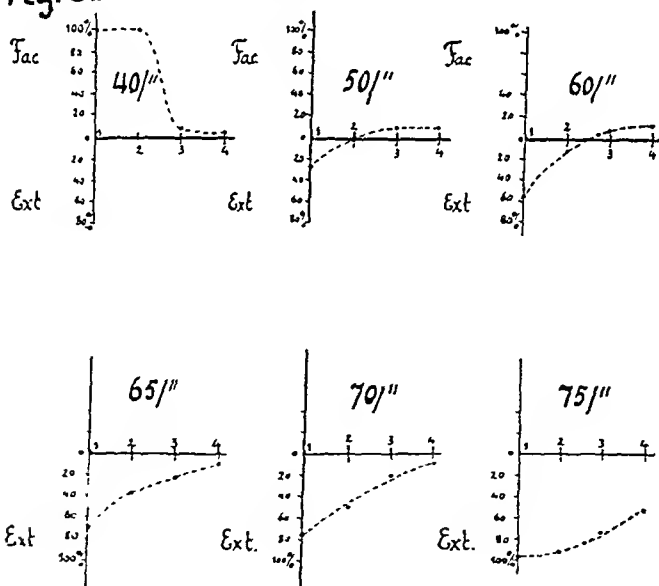
FIG. 6a. Feb. 21, 1935. *Macaca mulatta*. Dial-narcosis. Four stigmatic Ag-AgCl electrodes, 1.5 mm. apart, are so placed on a straight line on the "motor" arm area that the responses to stimulation of each of these foci have an element, extension of the wrist, in common. Record shows extinction from 1 on 1, equalization of response from 2 on 1, facilitation from 3 and from 4 on 1.

FIG. 6b. Same arrangement of electrodes as in Fig. 6a gives the average percentage change in size of many responses to test stimulation at focus 1 induced by antecedent stimulation at foci 1, 2, 3 and 4 respectively for various pattern-frequencies; all other parameters of stimulation and the interval between members of a pair being held constant throughout. At low frequencies pure facilitation in the vicinity of focus 1, at intermediate pattern-frequencies pure extinction around focus 1, facilitation at some distance (foci 3 and 4), at higher frequencies extinction from all foci, increasing as the frequency increases.

changing any of the parameters of stimulation, at constant interval, as a function of the separation of electrodes and the parameter varied. In the experiments of Fig. 6 the stimulations were at constant interval and the parameter varied was the pattern-frequency. On stimulation with lower pattern-frequencies facilitation was found greatest at focus 1 from focus 1, decreasing so as to be least at focus 1 from focus 4; with higher pattern-frequencies the same obtained for extinction. With intermediate pattern-frequencies extinction was found at focus 1 upon antecedent stimulation of near foci and facilitation upon antecedent stimulation of more distant foci. Figure 6A is an excerpt from a record with an intermediate pattern-frequency (60 per sec.); Fig. 6B shows diagrammatically this spatial distribution of facilitation and extinction combined as a function of the pattern-frequency of stimulation.

C. *Factors for Facilitation and Extinction*

The foregoing experiments have indicated the necessity of considering factors for facilitation and extinction, differing in kind and distribution. Inasmuch as these factors are functional changes in the CNS they can be disclosed only by examining what is going on *within* it. Since only changes in threshold and changes in activity can affect the response to test stimulation and since threshold and activity can both be affected by antecedent activity both must be investigated. Changes in the activity of any part of the CNS can be studied by recording the electrical activity of that part; changes in threshold of any part can be studied by determining the minimal electrical stimulation which produces an electrical response at that site.

It is essential to emphasize that, although these significant variables can be studied separately, they are inevitably interrelated in the living CNS. With impulses always impinging upon the part of the CNS under investigation a fall of threshold permits increased activity, increase of activity produces a rise of threshold. But that is not all. In peripheral nerve, changes in threshold have long been known to be associated with "after-potentials* and with changes in ion-concentration, notably phanges in pH. Therefore, in the following group of experiments the changes in activity and threshold were investigated by recording the electrical activity of various parts of the CNS, the threshold of the cortex, its "slow potentials"* and its pH before and after such electrical stimulation of the cortex as was shown to give facilitation or extinction of motor response to a particular test stimulation at a certain focus and after a given interval. It should be realized that in order to ascertain the altered electrical activity, "slow potentials" and pH of the cortex or other parts of the CNS at the time when the test stimulation would fall, this stimulation has to be omitted. Therefore, a separate, parallel experiment with antecedent *and* test stimulation has always to be made to ascertain whether, where and when facilitation or extinction obtains. With-

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* For criticism of this designation see Discussion, below.

out both experiments it is impossible to infer what and how distributed in space and time are the functional changes underlying facilitation and extinction.

One of the conclusions from the experiments represented in Fig. 6 was that one obtained facilitation or extinction depending upon the distance of the locus of the test stimulation from that of the antecedent stimulation. This means that one has to investigate the changes mentioned above in the immediate neighbourhood of stimulation, at foci in the vicinity, at distant foci in the cortex and finally at other levels of the CNS.

1. *Changes in electrical activity*

a. *In the immediate neighbourhood of cortical stimulation.* Electrical stimulation of the cortex such as to produce profound extinction results in a temporary diminution or silencing of the electrical activity at the site and in its immediate neighbourhood.[29] For optimal localization of stimulation and recording of the activity at the site of stimulation it is necessary to use for both a single pair of concentric electrodes. We have employed concentric Agar-Ag-AgCl electrodes, the central electrode ending in a glass capillary 0.1 to 0.3 mm. in diameter and the circumferential circular electrode, between concentric glass tubes, about 6 mm. in diameter and 1 mm. wide.* Figure 7 shows a cathode ray oscillogram of the electrical activity of the cortex before and immediately following extinguishing stimulation under these conditions, the D.C. amplifier being disconnected from the specimen during stimulation. It will be noted that the electrical cortical activity following this extinguishing stimulation, although subthreshold in regard to motor response, is greatly reduced. With electrical stimulation apt to produce motor afterdischarge (long pulses, *e.g.* 60~) one is likely to find immediately after the stimulation, even when subthreshold in regard to motor response, a short period of electrical afterdischarge followed by a longer period of diminished electrical activity of the cortex. Figure 8 illustrates this statement. The form of the spikes in the discharge indicates (as will be discussed later) that it started in the cortex nearer the central electrode and ended under the circumferential electrode, so that at this stage facilitation was present around the area of extinction under the central electrode (a situation comparable to graphs 2 and 3 of Fig. 6B), whereas a little later the cortex underneath both electrodes had passed over into the stage associated with extinction.

b. *In neighbouring foci.* For recording of changes in electrical activity several millimeters away from the locus of stimulation but still in the cytoarchitectonic area of the same subdivision of the sensorimotor cortex two pairs of electrodes can be used, thus obviating difficulties of interpretation. The phases of afterdischarge and diminution of activity, corresponding to facilitation and extinction respectively, are more prolonged and often more

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* These electrodes have been prepared for us by Dr. Nims and were found to be stable within 10μV.
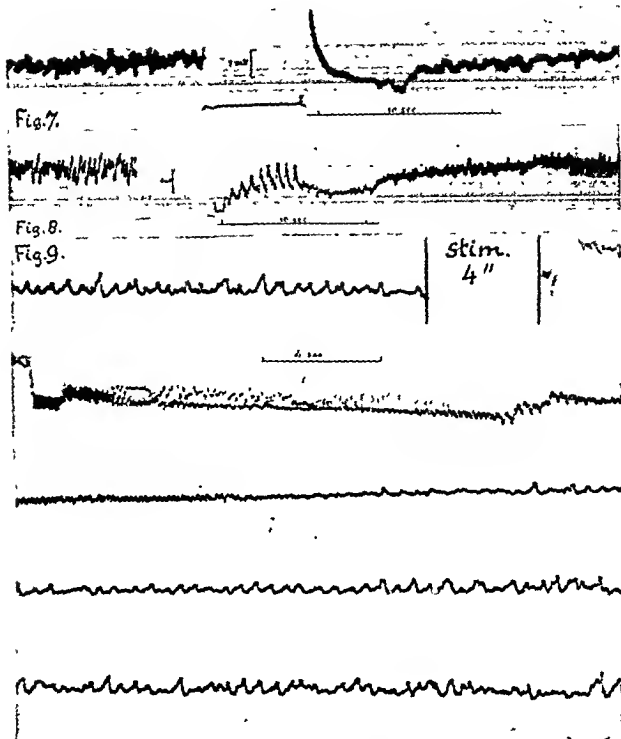
FIG. 7. Jan. 18, 1936 *Macaca mulatta*. Dial-narcosis. Cathode ray oscillogram of electrical activity of a focus of the "motor" arm area before and after electrical stimulation of this focus. Concentric Agar-Ag-AgCl electrodes used for stimulation and pick-up from a focus of motor arm area. Extinguishing subthreshold stimulation results in a very short-lived negative voltage drift followed by a positive drift and initial diminution of electrical activity of this focus Lower heavy white line shows period of stimulation, upper heavy white line shows absence of peripheral motor response.

FIG. 8. Jan. 17, 1936. Same arrangement of stimulation and pick-up electrodes as in Fig. 7. This extinguishing stimulation (60 ∼), known to give motor afterdischarge at or near threshold voltages, produced an initial electrical afterdischarge followed by diminution of electrical activity and gradual return to normal.

FIG. 9. Jan. 28, 1936. *Macaca mulatta*. Dial-narcosis. Cathode ray oscillogram of electrical activity of cortex before and after electrical stimulation. In this experiment two stimulating stigmatic Ag-AgCl electrodes were placed on dorsal portion of "motor" leg area (L.4) and parallel to them, 3.5 mm. distant, two similar pick-up electrodes on the ventral portion of the same area, thus minimizing voltage drifts. The subthreshold stimulation resulted in a marked prolonged electrical afterdischarge followed by diminution of electrical activity (extinction) with gradual return of it to normal. (Continuous record.)

pronounced. An example is supplied in Fig. 9 in which it will be seen that, following the electrical stimulation of a focus of L.4, the electrical activity of a focus of the same area 3.5 mm. distant is at first greatly increased—electrical afterdischarge—then diminished to return to normal about a minute after stimulation.

c. *In more remote parts of the cortex.* Under this heading comes the description of the changes in electrical activity in various cytoarchitectonic
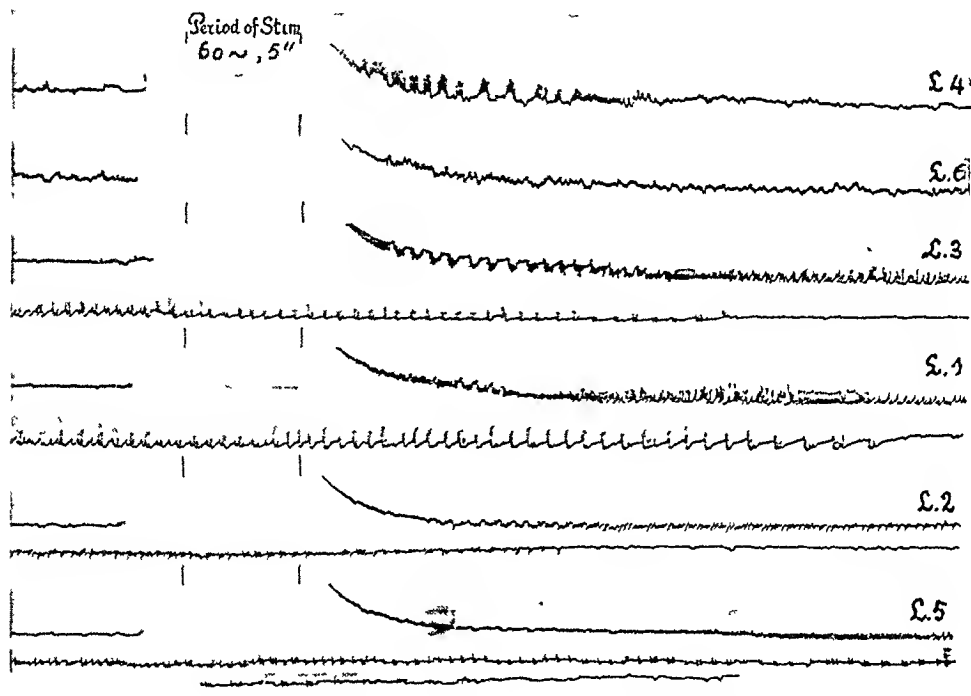
FIG. 10. April 23, 1936. *Macaca mulatta.* Dial-narcosis. Cathode ray oscillograms of electrical activity of various foci of the leg-subdivision of the sensorimotor cortex. In each of the records of this figure L.4 was stimulated (60~, 5 sec.) with bipolar Ag-AgCl electrodes and the electrical activity recorded with similar electrodes from the named areas successively. For further comments see text.

areas of one subdivision upon stimulation anywhere within this subdivision. With one pair of stimulating electrodes always on, let us say, L.4 the electrical activity of another locus in this area and of associated areas of this subdivision (L.6, L.3, L.1, L.2 and L.5) was recorded successively before and after stimulation. Figure 10 shows the changes in electrical activity in these various leg areas following stimulation of L.4. In the particular experiment to which Fig. 10 relates the pick-up electrodes were so arranged that those in the leg areas were successively connected to the grid of the amplifier, the cathode being simultaneously connected to an electrode in the corresponding area of the arm-subdivision. This arrangement allowed

one to interpret the sign of the recorded deflections in terms of the site and direction of propagation of the electrical activity in the leg subdivision, since the activity of the arm-subdivision was not influenced by the stimulation of L 4

In an experiment of this kind with a D C amplifier and intended to record slow ' D C potentials," the usual procedure of placing one of the electrodes as a reference electrode on a killed portion of the cortex is inappropriate on account of the spurious slow potentials produced

Examination of Fig 10 reveals the following a subliminal stimulation of L 4 results in electrical afterdischarge not only in L 4, but also in L 3, L 1, L 2 and L 5, not in L 6—a distribution of hyperactivity in entire harmony with the functional organization of the cortex as revealed by local strychninization,[14] b comparison of the pictures of after discharge in L 4 and L 3 shows that the sign of the rapid potential fluctuations in L 4 is always in the same direction (upwards = negative), whereas the fluctuations in L 3 are initially of the opposite sign, then suddenly reverse So far as we can see there is only one possible interpretation, compatible with our analysis of the strychnine spike,[15] namely that negative swings and negative spikes express activity originating in the area itself with the superficial layers leading, whereas the positive swings with positive spikes indicate activity with the deeper layers leading This interpretation is furthermore in harmony with the findings and interpretations of Bishop and collaborators,[2 3 4 5] of Marshall, Woolsey and Bard[6 7] and of Adrian [1] This indicates that L 4, which was stimulated electrically, is itself in active discharge immediately after this stimulation, whereas L 3 at first receives impulses from L 4 resulting only after more than 10 sec in an active discharge of the area itself Essentially the same can be seen in the other records of the postcentral areas and it is of interest to note that the active phase of the discharge begins later and ends later the more remote the area from L 4

In these experiments sub C 1, a, b and c the period of electrical afterdischarge is regularly followed by a period of electrical inactivity, as can be seen from Figs 8 and 9 This phenomenon is not obvious in Fig 10 because the animal was deeply narcotized and the amplification used was purposely small to insure full recording of the spikes of the after-discharge

d *In distant parts of the CNS* Associated changes in electrical activity can be observed in other portions of the CNS, e g the spinal cord [9] In Fig 11 is shown the electrical afterdischarge in the white matter of the lumbar enlargement (ventrolateral column) of a monkey's cord following electrical stimulation of a focus of the cortex of L 4 These four records were made with increasing voltage of stimulation, the last being just supraliminal Figure 12 is a record of the electrical activity of the grey matter (ventral horn) of the cervical enlargement of the cord before and after subliminal electrical stimulation of a cortical focus of A 4 Comparison of these two typical records shows that while the afterdischarge in the white matter resembles that obtainable from the cortex, the picture from the grey matter differs, in that
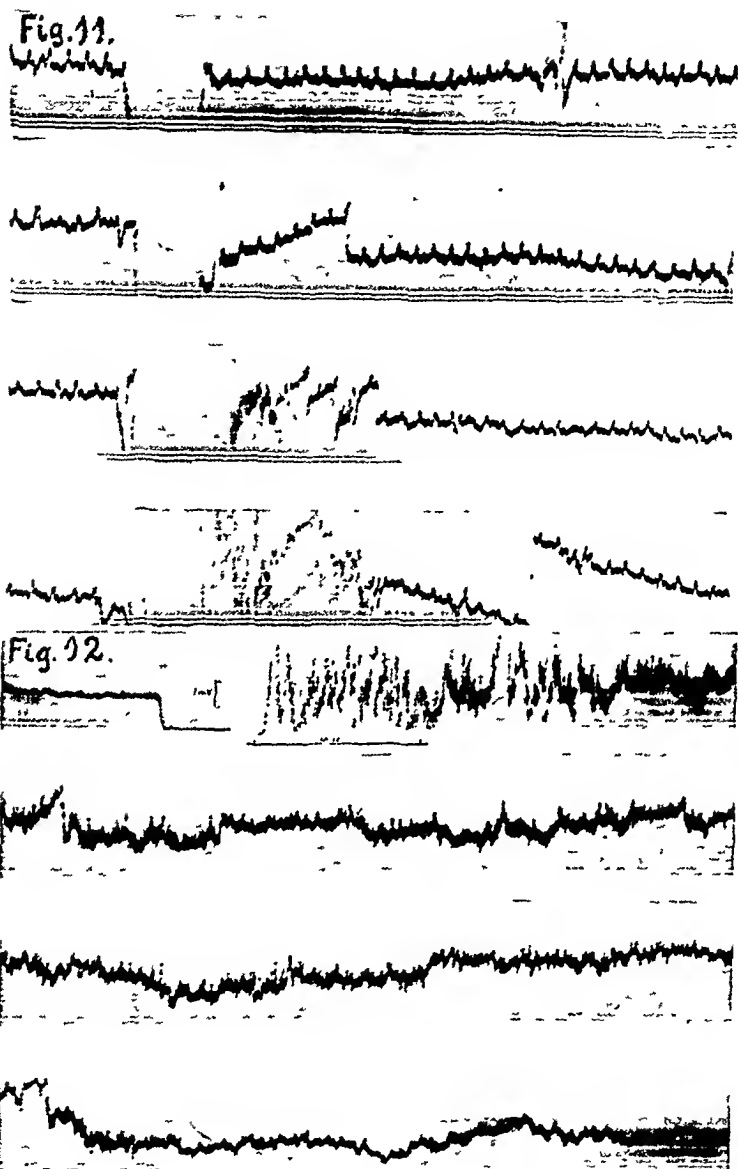
FIG. 11. Jan. 29, 1936. *Macaca mulatta.* Dial-narcosis. Cathode ray oscillograms of electrical activity of ventrolateral column of lumbar enlargement of spinal cord before and after electrical stimulation (60 ∿, 4 sec.) of contralateral motor leg area (L.4). Strength of stimulation in record 1 = 2000, in record 2 = 3000, in record 3 = 4000, in record 4 = 6000. The last stimulation was just supraliminal (see upper heavy white line in 4). Note voltage drifts so great as to require balancing of spot on screen of cathode ray oscillograph. Lower heavy white line in all records shows period of stimulation.

FIG. 12. Jan. 20, 1936. *Macaca mulatta.* Deep Dial-narcosis. Cathode ray oscillogram of electrical activity of ventral horn of eighth cervical segment of spinal cord before and after bipolar subthreshold stimulation of focus of contralateral "motor" arm area (A.4). Note the prolonged electrical afterdischarge in grey matter of cord. Under this deep narcosis extinction lasted more than 5 min., less than 10 min. (Continuous record.)

it is more irregular and much more prolonged. Once these changes in electrical activity following one period of stimulation of the cortex were known, their relation to facilitation and extinction could be investigated by applying at an appropriate interval a second, or test, stimulation. During the early stages of afterdischarge in a "motor" area (e.g. A.4) induced by stimulation of this area itself or of a remote related area (e.g. A.5) test stimulation to this "motor" area (A.4) results in a facilitated motor response, whereas during the later stages of afterdischarge this test stimulation elicits a smaller or no motor response (extinction) and even frequently terminates the existing afterdischarge. The motor response to test stimulation applied during the period of electrical inactivity of the cortex following an electrical afterdischarge is always extinguished.

2. Slow voltage drifts

When records of the electrical activity of the cortex before and after stimulation are taken with a direct-coupled amplifier the site of stimulation is seen to be negative at the end of stimulation and to become slowly positive with respect to a distant focus. These slow drifts appear with almost any type of electrode. With due precautions as to the type of electrodes and circuits used for pick-up and stimulation these changes can be shown to be not artifacts but physiological phenomena. To observe these changes as precisely as possible at the site of stimulation requires again that a single pair of concentric electrodes be used both for stimulation and pick-up. Obviously for such an experiment the electrodes must be non-polarizable; as such we used the concentric Agar-Ag-AgCl electrodes mentioned above, which in practice were found to be good to some 4 or $5\mu V$. It was with these electrodes that the record of Fig. 7, showing the negative (up) and positive (down) slow drifts at the site of stimulation, was obtained. For investigation of slow drifts at foci other than that stimulated separate pairs of electrodes for stimulation and pick-up can be used. This in itself obviates one of the possible sources of artifact, i.e. alteration of the pick-up electrodes by passage of the stimulating current. Moreover, one can use for stimulation a transformer, with a center-tap grounded and permanently connected to the cathode of the amplifier. For foci near the site of stimulation one of the pick-up electrodes, to wit, the "live" electrode, can be placed half-way between the two stimulating electrodes and connected to the grid of the amplifier, except during stimulation when it must be disconnected. The reference-electrode, placed on a distant unrelated cortical focus (i.e. one not affected by the stimulation) is connected permanently with the cathode of the amplifier, ground and the center-tap of the transformer. This circuit is so symmetrical as to be practically free of D.C. artifacts, except for a slight shift produced by rectification of the A.C.-current in the transformer. The slow voltage drifts recorded in this way are essentially similar to those already described; they are somewhat larger and more complicated by action potentials due to the greater separation of the electrodes. Moreover,
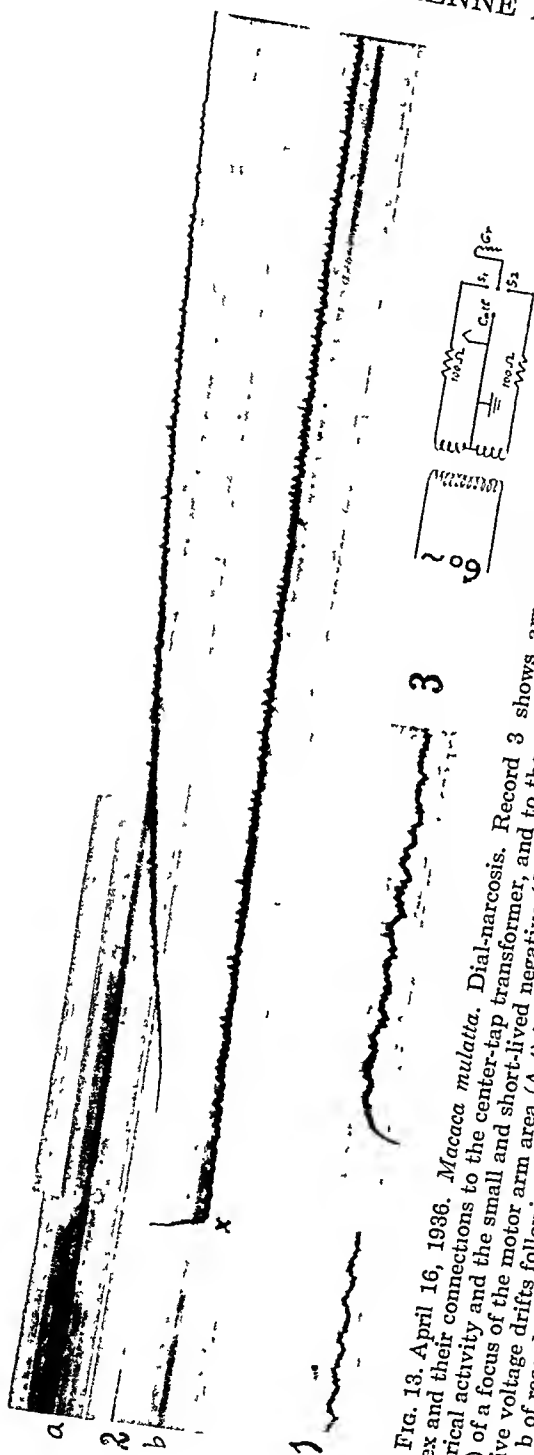
FIG. 13. April 16, 1936. *Macaca mulatta.* Dial-narcosis. Record 3 shows arrangement of four electrodes (Agar-Ag-AgCl) on cortex and their connections to the center-tap transformer, and to the cathode, ground and grid of the amplifier. Record 1 shows the electrical activity and the small and short-lived negative (down) and positive (up) voltage drifts following liminal stimulation (4 sec., 60~) of a focus of the motor arm area (A.4) in the lightly narcotized animal. Record 2 shows when the marked and prolonged negative and positive voltage drifts following liminal stimulation (4 sec., 60~) of the same focus when the animal was deeply narcotized. Sections a and b of record 2 continous. The insert in this record shows the interval at which equalization of threshold response occurred in this stage of narcosis. x = balancing of spot on screen.

inasmuch as the "live" lead is not exactly at the site of stimulation, they never show the complete inactivity (extinction) revealed with a single pair of concentric electrodes. Figure 13[3] shows diagrammatically the connections of the center-tap transformer and the amplifier to the four electrodes on the cortex. Figure 13[1] and 13[2] were obtained on the same animal, in both cases with liminal 60∼ stimulation. Figure 13[1] was obtained in the lightly narcotized monkey when facilitation lasted only a few seconds, whereas Fig. 13[2] was recorded under deep narcosis. It will be seen that in the latter case the stimulation is followed by a very marked negativity (downward) succeeded by a long lasting positivity (upward), so great as to require balancing of the spot on the screen of the cathode ray oscillograph at the point
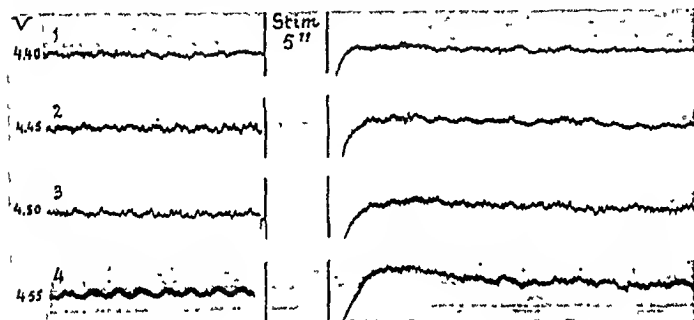
FIG. 14. April 15, 1936. *Macaca mulatta.* Light Dial-narcosis. These cathode ray oscillograms show the gradual increase of electrical afterdischarge and negative and positive voltage drifts with increase of stimulation of a focus of A.4. All stimulations (4 sec., 60∼) the same except for voltage. Arrangement of electrodes as in Fig 13. With these strengths of stimulation facilitation lasted as long as the periods of fast action potentials of these records. Voltages (V) at left of records.

marked x. On Fig. 13[2a] is an insert, from a record of a parallel experiment and taken with the same speed of paper, showing the interval at which repetition of the stimulation yields equal motor response. It will be observed that this happens at a time between the negativity and positivity when the voltage is practically zero. At all intervals shorter than this "equalization" interval facilitation of motor response was encountered; outside this interval for a period of many minutes, *i.e.* much longer than the record represents, total extinction of motor response obtained. Thus "negativity" is associated with facilitation, "positivity" with extinction. These voltage drifts are sufficiently prolonged to admit recording with a microvoltmeter[8] and an ordinary sensitive galvanometer, and by this method their sign and magnitude are found to be as indicated. Since the period of such a galvanometer is relatively long it distorts the time-relations and for this reason no such record is presented.

Such a simple association of these voltage drifts with facilitation and extinction is found only when electrical afterdischarge is avoided by proper selection of the stimulation. With afterdischarge, which during its stage of rapid discharge has been shown above to be a factor for facilitation, the period for facilitation is prolonged into the period of positivity. In the particular experiment of Fig. 14 wherein the afterdischarge had only a fast phase, the facilitation lasted as long as the afterdischarge, whereafter extinction, even to stronger test stimulation, ensued. Figure 14 demonstrates the changes in electrical activity and the slow drifts following four stimulations of a focus of A.4 at various voltages through capillary Agar-Ag-AgCl electrodes of high resistance. It will be seen that with gradual increase of the stimulating voltage the electrical afterdischarge and associated drifts become more pronounced. In all cases there was a motor afterdischarge of very small amplitude, though to the first two stimulations (records 1 and 2) there was no visible primary response.

It should be mentioned here that when an electrical afterdischarge is propagated into a remote area related to the one stimulated, this remote area at first "climbs" negative, and then, as it begins to discharge actively, "climbs" positive in respect to an unaffected reference-area, and when electrical inactivity ensues it is and remains for some time on the positive side. This means that the activity of a given area has the same effect on its slow voltage drifts regardless of how this activity was initiated, *i.e.* whether by direct electrical stimulation or by a disturbance propagated into it transsynaptically.

3. *Changes in pH of the cortex*

It has been shown so far that facilitation is associated with increased electrical activity and a negative voltage drift, extinction with decreased electrical activity and a positive voltage drift. These factors are sufficient to account for the period of facilitation, the time of equalization of motor response and the beginning of extinction. It was found, however, that the positive voltage drift disappeared before the electrical activity of the cortical focus returned and before extinction had passed off. Investigation of the latter part of the period of extinction showed that the end of extinction coincided with the return of normal electrical activity. The question then arose, what factor underlies the terminal portion of the period of electrical inactivity and associated extinction? Obviously this must be a change in threshold. Several old and new observations had pointed toward the pH as a significant variable in determining the activity of the nervous system, more particularly in determining the threshold of isolated nerve. Moreover, it was only reasonable to assume that the increased activity of the cortical cells stimulated would eventually lead to an increased production of acid metabolites. These considerations themselves were sufficient to necessitate pH-determinations of the cortex under various conditions of activity. For-

tunately this demand could be met, since the glass-electrode technique of pH-determinations had been developed sufficiently by Nims to be applicable to determinations *in vivo* and had been already used by him in collaborations with the authors in investigations on the effects of intravenous injections of acids, alkali and changes in ventilation on the pH of the cortex and the corresponding changes of its threshold.[16,17] In the second paper cited it was stated that a change of pH of the cortex from 7.3 to 7.5, however induced, resulted in a decrease of more than 25 per cent of the voltage required to initiate a minimal electrical afterdischarge, and that a decrease of pH to 7.1 raised the threshold much more than proportionally. In the same paper will be found evidence to the effect that hyperactivity of the cortex, whether due directly to electrical stimulation or to an afterdischarge propagated into an area investigated, causes a large decrease of pH which cannot be referred to anything but the increased activity of the nerve cells involved, and, finally, that this "acidity" persists as long as the prolonged diminution in electrical activity of the cortex and its concomitant rise in threshold[17] (pp. 286 and 287). In Fig. 7 of that paper an initial alkaline wave, beginning during stimulation, will be seen. At that time we were not certain that this wave might not be an artifact, although it was constantly present with all effective stimulations, regardless of the distance of the pH electrodes from the stimulating electrodes and notwithstanding all possible precautions to make the stimulating current symmetrical and the stimulating and recording circuits as independent as the requirements of the experiment permit. It was not until we had evidence of the association of an alkaline wave in the cortex with the rapid phase of a central electrical discharge, induced by convulsant drugs in the curarized animal,[18] that we were convinced of the significance of the observed initial alkalinity. Since this alkalinity coincides with the early part of the period of negative voltage drift it is impossible to observe the influence of this alkalinity by itself upon cortical threshold and motor response; one can only infer from the observed effect of cortical alkalinity otherwise induced that it must give a fall in threshold of all nerve cells in the "alkaline region" and so operate as a factor for facilitation.

Figure 15 presents diagrammatically the relation of facilitation and extinction of motor response induced by $60 \sim$ stimulation of a "motor" focus to the corresponding changes in electrical activity, voltage drifts and pH at the site of stimulation.

To summarize the results of the experiment sub C one can say 1. that increased activity, negative voltage drift and alkalinity operate as factors for facilitation; 2. that decreased activity, positive voltage drift and acidity operate as factors for extinction. The distribution of these factors in the CNS, both as regards time and place, is determined by the propagation of neural impulses from the site of initial stimulation, the increased activity in remote areas determining in them slow voltage drifts and changes in pH.
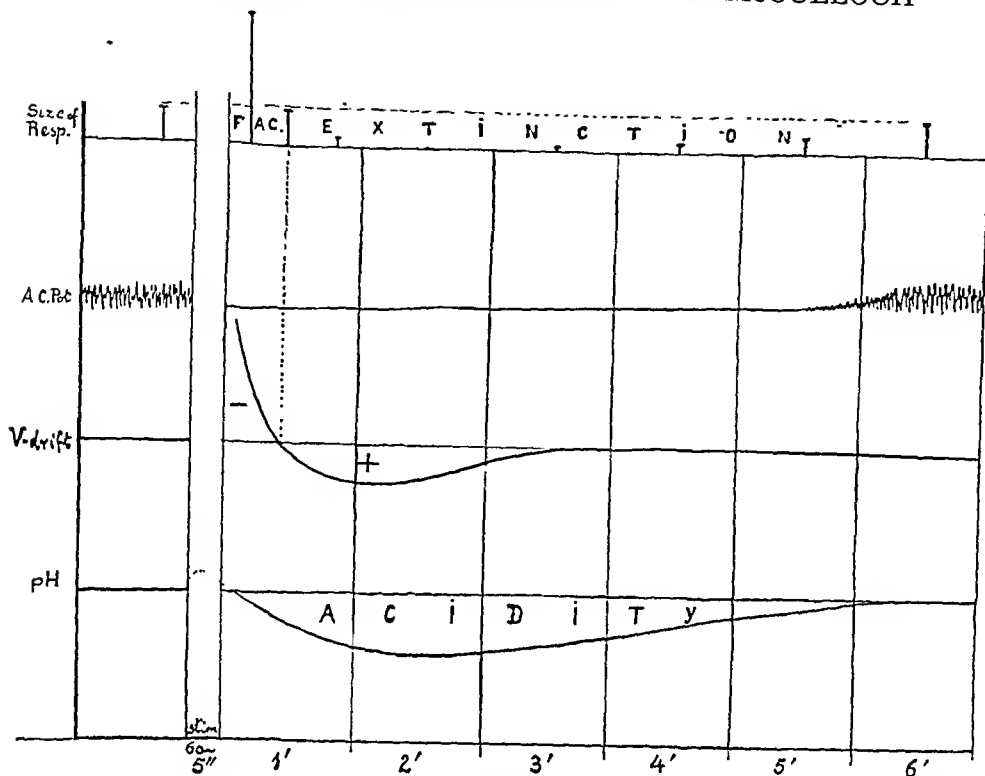
FIG. 15. Diagram of correlation of facilitation and extinction with changes in electrical activity, voltage drifts and pH at the site of stimulation.

D. *Mode of Action of Factors for Facilitation and Extinction*

From general considerations of a host of observations, old and new, of many investigators it seems permissible to infer that the mode of action of these three kinds of factors is as follows: 1. changes in activity as such alter the amount of summation to be expected at subsequent synapses; 2. slow voltage drifts are either the electrical manifestation of changes in temporospatially dispersed activity (*i.e.* action potentials which overlap and sum to give a smooth contour) or changes in voltage of cells that have been discharged (*i.e.* "after-potentials") or a mixture of these; 3. changes in pH involve all nervous structures lying within the region of altered pH, and thus involve cells that have not yet been discharged. These implications are of direct interest in connection with many of the problems pertaining to facilitation and extinction. First let us consider the dissociation of changes in latency and in amplitude, as exemplified in Fig. 4, where decrease in latency (facilitation) is associated with decrease in amplitude (extinction). To account for the decrease in latency of a response one must assume that less summation is required, *i.e.* that there is a fall in threshold to repetitive stimulation, whereas to account for the decrease in amplitude one must assume that fewer nerve cells are capable of responding.

1. *Relation of latency and threshold*

If the first of these assumptions is correct one must expect that stimulations giving facilitation with decrease in latency but with little or no change in amplitude will produce a marked fall in threshold of the focus under investigation. That this is so can be seen in Fig. 16. In this particular experiment the threshold was slightly below 4300, and 4200 was definitely subthreshold, yet the response to it as test stimulation was as large as the response to the antecedent stimulation at 4600. Even test stimulation at 3500, *i.e.* following a stimulation at 4600, brought in a just visible response. Figure 16A, therefore, shows that some cells of the nervous structures involved
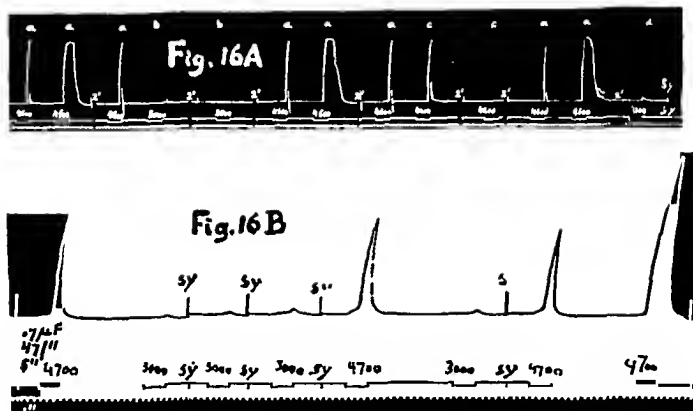
FIG. 16A. Dec. 1, 1938. *Macaca mulatta*. Dial-narcosis. This figure shows that facilitation as judged by decrease of latency is associated with a marked fall of the voltage threshold.

FIG. 16B. March 10, 1939. *Macaca mulatta*. Dial-narcosis. This figure shows constancy of threshold with constancy of latency accompanied by marked increase of amplitude (see last two responses).

have been rendered excitable to voltages formerly far subthreshold. It is even possible to dissociate latency and amplitude in the opposite direction, *i.e.* to obtain facilitation as judged by increase of amplitude without change of latency or even with a slight increase of latency (see Fig. 16B). Under these conditions there is no obvious change or a slight rise in voltage-threshold. Taken together these findings show that latency and voltage-threshold are covariant regardless of amplitude. Amplitude of response must, therefore, depend upon some factor other than threshold; this must be a change in activity available for summation.

2. *Dissociation of latency and amplitude of motor response*

The second assumption, namely that during extinction, as judged by decreased amplitude, fewer cells are capable of responding, implies that these cells have passed over into a period of extinction, and this, in turn, that with pairs of equal stimulation the amplitude should decrease with increase of interval because cells previously active or in the period of lowered threshold (negative voltage drift) will now have passed into the period of raised threshold (positive voltage drift), even when the decrease of latency is not yet materially affected by the increase of interval. That this is the case is seen in Fig. 17. This figure shows a series of eight pairs of equal stimulations with gradually increasing intervals and so exhibits the latency and amplitude of the second response as a function of the interval. It will be seen that the latencies of the second responses are smaller than those of the first in graphs a, b, c and d, equal in e, and greater in f, g and h, though the amplitude of the second responses is decreased in *all* graphs, *i.e.* the amplitude falls off earlier than the decrease in latency.

It should be noted that between each graph of Fig. 17 there was a pause of 2 minutes. The variations in size of the first responses are the expression of the waves of cortical excitability previously described.[6, 23]

FIG. 17. Oct. 16, 1936. *Macaca mulatta.* Dial-narcosis. This figure shows the dissociation of latency and amplitude in the response to constant test stimulation as a function of the interval after constant antecedent stimulation. Intermission between pairs ≈ 2 min. Stimulus selected to avoid afterdischarge.

Thus in the first half of Fig. 17 one has an admixture of facilitation and extinction, in the latter half pure extinction, *i.e.* by both criteria. In order to obtain such a record one has to use relatively long stimulation and one not giving cortical afterdischarge, so that at the end of it many nerve cells have been discharged so often as to suffer the rise in threshold associated with positivity. In graph a, with an interval of only 0.2 of a second, the response to the second stimulation is practically a continuation of that to the first and declines during stimulation. Such a pair of stimulations may well be regarded as a single stimulation of double duration, the response to which exhibits extinction during stimulation. To account for this, one has to suppose that all the nerve cells which stimulation at this site, of this voltage and this wave form (pulse-frequency) can reach have become inexcitable to it. At such a time a response can still be elicited by stimulation of higher voltage or longer wave form (lower pulse-frequency), but not by one with higher pattern-frequency. Here it is important to point out a similarity to

peripheral nerve, in that, so long as afterdischarge is avoided, there exists for cortical stimulation a maximal stimulus in terms of change of any one parameter of stimulation except the voltage. The experiment just cited (Fig. 17) exhibits the justification of this statement in regard to the duration of stimulation. The exception concerning the voltage depends obviously upon the limited number of fibers in a peripheral nerve, whereas in the case of the cortex a higher voltage with consequent wider spread of current will always find more remote cells hitherto uninvolved.

3. *Influence of parameters of stimulation*

In a previous paper, dealing with pairs of equal stimulation, it was pointed out that, other conditions being equal, an increase in the total energy of stimulation by increase of any one of its parameters (duration, voltage, pattern-frequency or pulse-frequency) gave increase extinction[13] (p. 520). In that same paper it was further stated that with pairs of *unequal* stimulations the change in size of the motor response to the test stimulation is determined by each and every one of the parameters of both the antecedent and the test stimulation, as well, of course, as by their interval.

Though we cannot as yet show the relation of each of these nine variables to the temporo-spatial distribution of the three known factors for facilitation and extinction, we have now to present some findings in such experiments with pairs of unequal stimulations.

a. *Pairs unequal in duration only.* In the experiment of Fig. 18A four pairs of stimulations, the interval between members of each pair being 15 sec., were
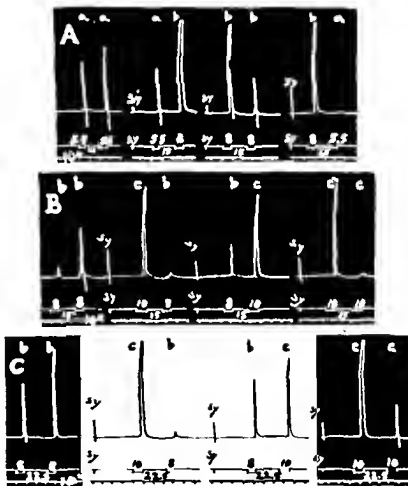
FIG. 18. Dec. 2, 1938. *Macaca mulatta.* Dial-narcosis. Each record contains four pairs of stimulations, differing only in duration. Unchanged parameters of these stimulations: 40/"; .5μF; VD = 2150. The interval between members of each pair was in records A and B 15 sec., and in record C 22.5 sec. The durations in record A were 5.5 and 8 sec.; in records B and C, 8 and 10 sec. Note in record A that "a" facilitates "a" and "b", "b" extinguishes "b" and "a". In record B, "b" facilitates "b", facilitates "c" as to latency, extinguishes it as to amplitude, "c" extinguishes "b" and "c". In record C it will be seen that "b" facilitates "b", but extinguishes "c", while "c" extinguishes both "b" and "c".

given. It will be seen that the shorter stimulation, "a", always produced facilitation, the longer, "b", always extinction; for the unaffected size of the response to "b" see responses 5 and 7. From this series it might seem as if the duration of the test stimulation is not significant; however, from Fig. 18B, where longer stimulations (8 and 10 sec. respectively) were used, it will be seen that the duration of the test stimulation must be considered. While the long stimulation, "c", extinguishes both the response to itself, "c", and to the short stimulation, "b", and this stimulation, "b", facilitates the response to itself, "b", it produces a mixture of facilitation (shorter latency) and extinction (lesser amplitude) of the response to stimulation "c". Records A and B were taken with the same interval (15 sec.) between members of each pair of stimulations.

In Fig. 18C, with the same type of stimulation as in Fig. 18B, but with the interval increased to 22.5 sec., it will be seen that now the effect of "b" on the response to "c" is extinction with regard to both latency and amplitude. The differences in results of Figs. 18B and 18C are in entire harmony with those of Fig. 17, showing the relation of the interval to the dissociation of latency and amplitude of the second response. The results of Fig. 18A and 18B, due to difference in duration of the stimulations ("a" and "b" versus "b" and "c"), are to be expected because the shortest of these, "a", is such as to give predominantly facilitation, whereas "b", being longer, produces somewhat more extinction, thus resulting in the admixture of facilitation and extinction (see Fig. 18B, pair "bc"), present also in Fig. 17.

In records like that of Fig. 18C, where one pair of stimulations produces one result and the other three the opposite, one is forced to conclude that the altered parameter of stimulation is significant in both antecedent and test stimulation. In the experiment of Fig. 18C it was the change in duration; in the subsequent Fig. 19, 20 and 21 the same will be shown for each of the other parameters.

b. *Pairs unequal in pattern-frequency only.* In experiments like the one depicted in Fig. 19 the only difference between the members of a pair of
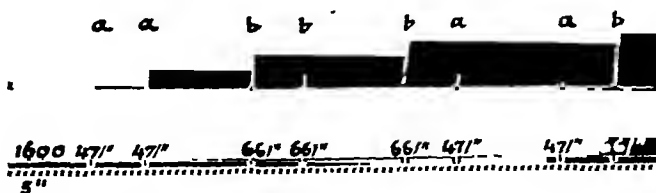
FIG. 19. Feb. 12, 1935. *Macaca mulatta.* Dial-narcosis. This figure illustrates the effect of change in pattern-frequency alone. "a" facilitates "a" and "b"; "b" facilitates "a", but extinguishes "b".

stimulations was in the number of pulses per second (pattern-frequency)· By suitable selection of all parameters of the two stimulations and their interval it is possible to find such stimulations that those of lower pattern-

frequency produce facilitation of response to themselves and to those of higher frequency, whereas those of higher pattern frequency facilitate responses to those of lower frequency in spite of the fact that they produce extinction to themselves

That in the pair "ba" the response to "a" is facilitated is clear by comparing this response with the first and seventh responses, which are unaffected by antecedent stimulation This relation of these responses shows that the pattern frequencies of both antecedent and test stimulation are significant

  c *Pairs unequal in pulse-frequency only* Figure 20 represents an experiment in which only the pulse-duration is altered The stimulation with longer pulses, i e of lower pulse-frequency, always extinguishes, whereas stimulation with shorter pulses, i e of higher pulse-frequency, while it facilitates the response to itself, extinguishes the response to the stimulation of lower pulse-frequency

That in the pair "ab" the response to "b" is extinguished is clear by
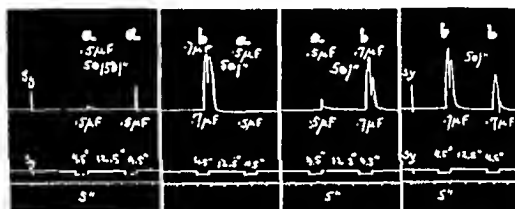
Fig 20 Feb 21, 1939 *Macaca mulatta* Dial narcosis This figure demonstrates influence of change in pulse frequency alone "a" facilitates ' a", but extinguishes "b", "b ' extinguishes "a" and ' b"

comparing it with the third and seventh responses, which are unaffected by antecedent stimulation Looking at this figure it is at once apparent that when all other parameters of stimulation are held constant, that having the lower pulse frequency produces the larger response, i e that more motor-units have been excited This presumably means that more cortical cells have been excited, rather than that a given group has been excited more often, for the pattern frequency and duration of the stimulation were held constant Since this experiment was made at constant voltage also, the voltage-gradient at any point in the cortex remains constant from stimulation to stimulation Therefore, the increase in response with decrease of pulse frequency is presumably due to an increase in the percentage of nerve cells excited within the area having a given voltage-gradient, rather than to any increase of that area If this conception is correct, it must mean that the longer pulse-form excites a larger percentage of the cells by inclusion of cells with higher threshold This is borne out by the comparatively slight effect of shorter

pulses upon the responses to longer pulses, and the dominance of stimulation with longer pulses; for these can excite all the cells which the short pulses had excited, certainly if their threshold were lowered and even if it were somewhat raised. On the other hand it also implies that with longer pulses which can excite the vast majority of the nerve cells the decrease of excitability of a few of these will be sufficient to exhibit decrease of amplitude of response (extinction), whereas with shorter pulses there always remain enough nerve cells available for increase of amplitude of response (facilitation) even though many cells originally discharged have become inexcitable to these or longer pulses. Thus it should be possible to find a stimulation of shorter pulse-form which, though it produces facilitation of response to an equal test stimulation, produces extinction to test stimulation of longer pulse-form, as shown indeed in Fig. 20. Compare again the facilitation in record 1 and the extinction of response to "b" by "a" in record 3 using the response to "b" in record 2 or the first response to "b" in record 4 as standard.
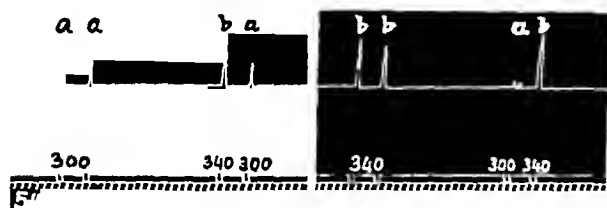
FIG. 21. Feb. 14, 1935. *Macaca mulatta.* Dialnarcosis. This figure shows the effect of change in voltage alone. "a" facilitates "a" and "b"; "b" facilitates "a", but extinguishes "b".

d. *Pairs unequal in voltage only.* Figure 21 presents an experiment in which only the voltage is altered. The stimulation of lower voltage always facilitates, that of higher voltage, while it facilitates the response to stimulation of lower voltage, extinguishes the response to itself. This finding is in entire harmony with that presented in Fig. 16, in which stimulation at the higher voltage, which failed to produce facilitation as judged by increase of amplitude of response to itself, brought in a response to voltages previously subliminal.

That in the pair "ba" the response to "a" is facilitated is clear by comparing this response with the first and seventh responses, which are unaffected by antecedent stimulation.

In experiments wherein the voltage of stimulation is changed there is inherent a complexity not encountered in experiments with other inequalities of stimulation at a single focus. For not only is there with increase of voltage an increase in the percentage of nerve cells stimulated in the area reached by stimulation with lower voltage, but also an increase of the area affected (greater spread of current). To which of these an observed change of response is to be referred is, of course, uncertain, which precludes further analysis.

The changes in the population of nerve cells directly excited when voltage or pulse-form is changed, is a complexity absent when only pattern-frequency or duration is changed. Since with changes of one of the latter

parameters alone the same population of nerve cells is excited—either more frequently by higher pattern-frequency or more often by longer duration—changes of threshold become of paramount importance and the results referable to the factors for facilitation and extinction.

4. *Triple stimulation*

At this point the findings with triple stimulation (see Fig. 3) must be considered in conjunction with parallel experiments on electrical activity and slow voltage drifts. The significant finding in such parallel experiments is that whereas the first stimulation initiates electrical afterdischarge and negative voltage drift followed by positive voltage drift, the second stimulation elicits no electrical afterdischarge but does produce a negative voltage drift which is consistently associated with decreased latency of the third (facilitated) response, irrespective of the amplitude of this response. This confirms one in the opinion that facilitation as expressed by decrease of latency of response is associated with decrease in threshold to repetitive stimulation, rather than with involvement of new neurones, excited transsynaptically by afterdischarge, which by summation is unquestionably a factor for increase of amplitude.

E. *Predictions*
1. *Conditions for reversal of a cortical focus*

All the facts and considerations enumerated above led to certain predictions concerning the apparent reversal of a cortical focus by antecedent stimulation of a related antagonistic focus. The predictions were that, irrespective of whether the stimulation of the focus to be reversed was predominantly facilitating or extinguishing at the interval under investigation, the reversal would occur provided 1. that the antecedent stimulation of the antagonistic focus produced strong primary facilitation at the interval in question; 2. that the primary facilitation of this antagonistic focus be such as to exhibit increase of amplitude or afterdischarge and not merely decrease of latency.

This reversal of a cortical focus was designated above as an apparent reversal because the evidence presented led to the conclusion that the reversal does not depend upon any change at the focus reversed nor even upon a fall in threshold of the antagonistic focus, but upon persistence of activity involving lower levels of the CNS, *e.g.* the spinal cord. A reversal of response in which the cortex certainly plays a significant rôle has been observed with relatively prolonged stimulation of a single focus so as to extinguish it and facilitate a neighbouring antagonistic focus. These predictions have been verified by the following results, all of which can be seen in one figure, namely Fig. 22. This figure presents six records, each consisting of four pairs of stimulations to two cortical foci, 3 mm. apart, one for extension (E) and one for flexion (F) at the elbow.

The antagonistic movements of the forearm at this joint were recorded with two tambours, the levers of which were attached with threads to the forearm just above the wrist. In the resting position these threads were just slack with the intention of obtaining independent records of the two mo-
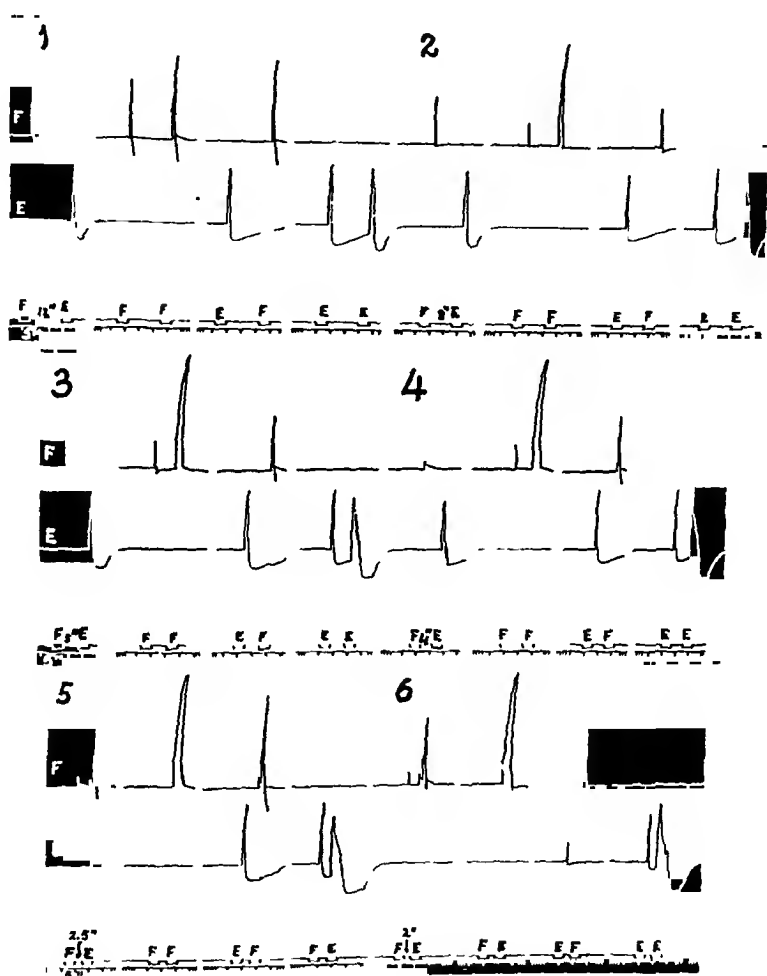
FIG. 22. Jan. 26, 1937. *Macaca mulatta.* Dial-narcosis. This figure shows records of flexion and extension at elbow upon cortical stimulation of a flexor and an extensor focus respectively. In record 5 the appearance of a flexor response to E stimulation will be seen; for complete reversal see first graph of record 6. For further details see text. All parameters of stimulation constant throughout; only interval gradually shortened.

tions. The excursions of the levers were recorded on the kymograph by means of two tambours, the upper recording the flexion, the lower the extension at the elbow. All stimulations were kept constant throughout the

experiment and only the interval was decreased from record to record. The parameters of stimulation were chosen to produce primary facilitation at all intervals at the F focus, extinction with long and facilitation with very short intervals at the E focus.

Complete reversal can be seen in the first pair of responses (FE) of record 6, where stimulation of E 2 sec. after stimulation of F results in flexion only. This reversal is not obtained with antecedent extinguishing stimulation of the antagonistic focus (see pairs EF and EE of record 1), nor with weakly facilitating stimulation (see pairs FE and FF of records 1, 2, 3 and 4), but only with strongly facilitating stimulation (pairs FE and FF of records 5 and 6). Nor is facilitation as judged by decrease in latency alone sufficient to produce reversal (pairs EF and EE of records 2 through 6) even when, as in record 6, the latency is reduced to a minimum.

Thus, these findings fulfil the predictions that in order to obtain the apparent reversal of a cortical focus the stimulation of the antagonistic focus must be such as to produce primary facilitation, *i.e.* increased responsiveness to repetition of stimulation at that same focus, as judged by increase of amplitude (usually accompanied by motor afterdischarge) at the interval in question.

Scrutiny of these records brings out another interesting relation of the E and F responses. Reversal of a cortical focus necessarily includes diminution of the normal response to stimulation of the "reversed" focus, as shown by the gradual decline of the E responses in pairs FE in all records of Fig. 22 (as well as the appearance of the F response to the E stimulation). This diminution cannot be considered as an extinction of this response, but must be interpreted as an *inhibition* in the extensor systems due to activity in the reciprocally related flexor systems, which finally in records 5 and 6 produce the F response to E stimulation. But that is not all. The E stimulation is predominantly extinguishing reaching its maximal effectiveness at an interval of 2.5 sec. The underlying decrease in activity of the extensor systems (extinction) should then manifest itself as an increased response to the F stimulation. That such is the case is seen in the increase in the F responses in the EF pairs of records 1 through 5. The decrease of this same response in record 6 at an interval of 2 sec. is caused by the appearance of some facilitation in the extensor systems at this short interval, seen in EE of this record.

One more point in regard to Fig. 22 should be noted. The stimulation to the F focus being facilitating, and the E stimulation not, there is always more excitation in the flexor than in the extensor systems. With decrease in interval and the eventual appearance of flexor response to the E stimulation this increased activity of the flexor system results in extinction in that system, as evidenced by the gradual decline, from record to record, in the size of the first response in each FF pair and their eventual disappearance in records 5 and 6. It is well to take this point into consideration in judging the amount of facilitation in these pairs in the successive records of Fig. 22.

Fig. 25

Fig. 26

Fig. 27

Fig. 28

Fig. 29
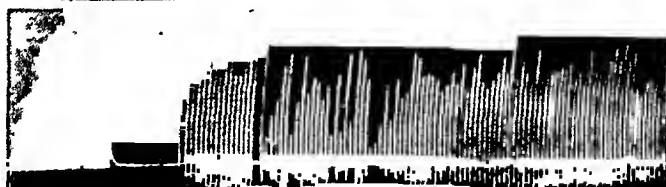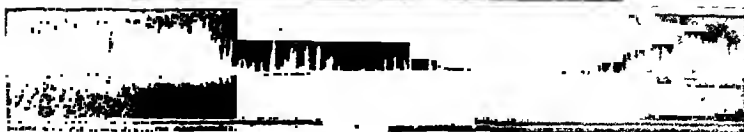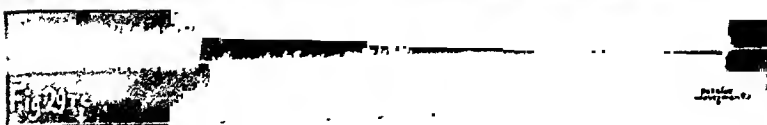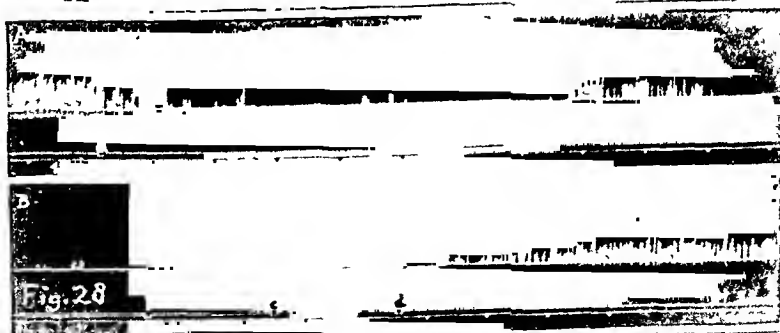
FIGS. 23–29. (*For legends see opposite page.*)

2. *Changes in the knee-jerk*

Consideration of the above findings with the observations of long lasting changes in activity induced in the spinal cord by cortical stimulation (see sub C.1.d.), led to several predictions as to the effect of cortical stimulation on "spinal" reflexes. To test these predictions the knee-jerk (KJ) was elicited periodically[19] and changes in its amplitude induced by cortical stimulation recorded kymographically. The predictions were the following: 1. facilitating stimulation of a cortical focus for extension at the knee will produce a long lasting facilitation of the KJ; 2. extinguishing stimulation of the same focus will produce a prolonged diminution (extinction) of the KJ; 3. facilitating stimulation of an antagonistic cortical focus, yielding flexion at the knee, will result in a prolonged diminution (inhibition) of the KJ; 4. extinguishing stimulation of this same focus will enhance the KJ.

Inasmuch as this enhancement is due to factors for extinction in antagonistic systems we would avoid the term facilitation

As corollaries to the foregoing it can be predicted that facilitating stimulation of an extensor focus and extinguishing stimulation of a flexor focus should bring back the KJ when it has been obliterated either by extinguishing stimulation of an extensor focus or facilitating stimulation of a flexor focus, and finally that passive movements at the knee will bring back by facilitation an extinguished or inhibited KJ.

Figure 23 shows the temporary *facilitation* of the KJ following facilitating stimulation of a focus for extension at the knee. Figure 24 shows the temporary *extinction* of the KJ following extinguishing stimulation of a focus for extension at the knee. Figure 25 shows the temporary *inhibition* of the KJ following facilitating stimulation of a focus for flexion at the knee.

Fig. 23 Feb 18, 1937 *Mococo mulatto* Dial-narcosis *Facilitation* of knee-jerk by *facilitating* stimulation (0 3μF, 40/″, 3 sec , VD =5500) of a focus of L 4 for *extension* at the knee

Fig 24 Nov 17, 1938 *Macaco mulatta* Dial narcosis *Extinction* of knee jerk by *extinguishing* stimulation (0 6μF, 80/″, 6 sec , VD =1000) of a L 4 focus for *extension* at the knee

Fig 25 Nov 17, 1938 Same animal as of Fig 22 *Inhibition* of knee-jerk by *facilitating* stimulation (0 3μF, 30/″, 4 sec , VD =9000) of a L 4 focus for *flexion* at the knee

Fig 26 Nov 8, 1938 *Macaca mulatta*. Dial-narcosis KJ elicited every 2 sec *Enhancement* of KJ by *extinguishing* stimulation (1μF, 80/″, 6 sec , VD =500) of an L 4 focus for *flexion* at the knee

Fig. 27. Nov. 17, 1938 *Macaca mulatta* Dial-narcosis KJ *extinguished* by *extinction* of an L 4 focus for *extension* at knee KJ *returns* after *facilitating* stimulation of same focus

Fig 28 Nov. 17, 1938 Same animal as of Fig 25 In record A *inhibition* of KJ after *facilitating* stimulation (0 3μF, 30/″, 4 sec , VD =7000) of a L 4 focus for *flexion* at knee In record B *return* of KJ after repeated *facilitating* stimulation (1μF, 80/″, 6 sec , VD =1000) of *same* focus

Fig 29 Nov. 21, 1938 *Macaca mulatta* Dial narcosis Disappearance of KJ after *thermocoagulation* at 80°C for 5 seconds of the area (3 mm ²)of L 4 from which *extension* at knee could be elicited The KJ was absent for 15 hours and could then be brought back only by often repeated passive movements of leg at knee and even then its amplitude was small and irregular.

Figure 26 shows the temporary *enhancement** of the KJ following extinguishing stimulation of a focus for flexion at the knee. Figure 27 shows the return of the KJ by repeated facilitating stimulation of that focus for extension at the knee, extinguishing stimulation of which had extinguished the KJ. Figure 28 shows the return of the KJ by repeated extinguishing stimulation of that focus for flexion at the knee, facilitating stimulation of which had inhibited the KJ.

Each of the above predictions has, therefore, been verified. These findings in the KJ beautifully illustrate the importance of a proper balance of these reciprocally related flexor and extensor systems in the maintenance of the normal KJ. Obviously to these systems belong those flexor and extensor foci in the cortex, the stimulation of which, as shown above, profoundly changes the KJ. It became of interest, therefore, to investigate whether the destruction of one of these antagonistically, or reciprocally, related foci, leaving the other intact, would not so upset the balance of these systems as to alter the KJ demonstrably.

To answer this question the cortical area for extension at the knee of a monkey was mapped and found to be *ca.* 2 mm. in diameter. Extinguishing stimulation of the center of this area resulted in typical extinction of the KJ, which was reinitiated by passive flexions and extensions at the knee. After the KJ had returned to normal this extensor area was precisely thermocoagulated (80°C for 6 sec.), which after 2 min. resulted in an abrupt and almost complete obliteration of the KJ which became complete at the end of 4 min. Attempts to reinitiate it by passive movements at the knee resulted only in a few rapidly declining KJs (see Fig. 29). Extinguishing stimulation of the adjacent intact cortical flexor focus although resulting in prompt flexor responses at the knee failed to bring back the obliterated KJ. Fifteen hours after this thermocoagulation the KJ was still absent and when restored, after many attempts at facilitation by passive movements at the knee, was small and irregular. This experiment shows that in the monkey, even under narcosis, cortical impulses impinge upon the spinal cord and that cessation of impulses arising in a cortical focus for extension at the knee is sufficient to produce an imbalance between the extensor and flexor systems thus obliterating the KJ.

That it is a specific imbalance between the extensor and flexor systems rather than the elimination of non-specific cortico-spinal impulses, maintaining the general level of activity in the spinal cord, follows from the observation that thermocoagulation of the whole precentral leg area (L.4, L.4-S and L.6) results only in a very transient diminution of the KJ for *circa* 5 minutes, after which it returns to its original amplitude. The finding that a very circumscribed cortical lesion results in the loss of a reflex usually considered spinal and that an extensive destructive lesion of the cortex, including this area, does not result in this disorder is obviously of interest in regard to the problem of functional localization in the cortex.

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* For the choice of this designation see p. 347.

DISCUSSION

Before entering into the discussion it seems advisable to sum up the main points of the results presented. The first point is that it is now established that underlying facilitation and extinction are changes in the CNS induced by antecedent activity, not by mere passage of current. Second, that these changes differ in kind, in time and in space. Third, that they can operate only by affecting threshold and (or) activity and that in the living CNS a change in either of these inevitably induces a change in the other.

We have isolated for study the electrical activity, the slow potentials and the pH and examined these at the site of stimulation and at remote parts of the CNS, and correlated them with facilitation and extinction. This analysis has shown that increased activity, increased excitability associated with negative voltage drift and a probable alkalinity are factors for facilitation, that decreased activity, decreased excitability associated with positive voltage drift and acidity are factors for extinction. On the basis of these findings it was possible to explain some of the findings with pairs of unequal stimulations and to predict and verify conditions for "reversal" of a cortical focus and for changes in the knee-jerk induced by cortical stimulation.

The following points need more detailed consideration: (i) as stated in a previous paper (13, p. 523) it was Lorente de Nó who suggested that the diminution or absence of response to test stimulation might result from an absence of background-activity in reverberating circuits, because they have been forced to discharge simultaneously and, therefore, have become simultaneously refractory. The first part of this suggestion has been more than substantiated; for the rapid phase of an electrical afterdischarge is always associated with facilitation, the diminution or absence of electrical activity following hyperactivity, whether due to direct stimulation or to an afterdischarge propagated transsynaptically to the area under investigation, is always associated with extinction. Our technique has not been such as to allow verification of the second part of Lorente de Nó's suggestion, except for this, that stimulation during the latter part of a prolonged electrical afterdischarge regularly terminates it.

In this connection it is of interest to examine the typical form of an electrical afterdischarge as exemplified in Fig. 10 in the record from area L.3. Following the long period during which this area has received impulses from the area stimulated (L.4) the active discharge of the area itself is characterized by a burst of rapid electrical fluctuations of remarkably constant form and interval; these end abruptly and are followed by much larger fluctuations of an entirely different form and lower frequency. This type of change may be repeated several times during a single afterdischarge until the frequency has dropped to about 3 per sec. The end of the discharge may exhibit a gradual decline in voltage (as in Fig. 10), but often ends abruptly, in either case followed by diminution of electrical activity. This sudden drop in frequency associated with a change in wave form is what one would expect from Lorente de Nó's work[22-25] for short chains being discharged

more frequently than long chains will sooner suffer a rise in threshold and cease to conduct, leaving only the longer circuits active.

This interpretation receives support from experiments on electrical afterdischarge in monkeys before and after deep undercutting of the cortex. For, following such a lesion the picture of afterdischarge is unchanged except for the absence of the slower, about 3 per sec. rhythms. This indicates that the circuits for the higher frequencies of afterdischarge are purely cortical or cortico-cortical, whereas those for the low frequencies are relayed back to the cortex by subcortical or even lower structures.

This analysis explains why one finds extinction, not facilitation, during the slow, terminal phase of an afterdischarge, for at this time too many cells have become inexcitable. As one would expect from this and as stated on p. 331 this latter part of an electrical afterdischarge is associated with a positive voltage drift.

(ii). This brings us to the slow voltage changes that follow prolonged enforced activity. We not not wish to call these phenomena after-potentials; first, because, strictly speaking, they are not potentials in the sense of the physicist, and second, because the conditions for experimentation are not those for which an after-potential is defined, namely as alteration in the voltage of a current of injury (*positive Nachschwankung* of Ewald Hering[23]) in excised nerve. Nor do we wish to use Gasser's designation[22] for somewhat similar phenomena in the spinal cord, which he calls intermediary potentials, not only because this again introduces the notion of potentials, but also because it refers the phenomena at hand to particular structures, namely internuncial neurones.

We are indebted to Dr. C. H. Prescott of the Bell Telephone Laboratories for calling to our attention that an electrical potential is not defined in the presence of a chemical reaction or where for any other reason there is a change in the carrier of the current, *i.e.* from an ion in solution to an electron in a wire or *vice versa*. What one observes under these conditions, *i.e. in all ordinary electrophysiological experiments,* are, strictly speaking, voltages, not potentials. For these reasons the designation "voltage drifts" seems to us the simplest, appropriate and noncommittal description. The term "drift" seems the more appropriate because these slow changes in voltage are presumably not the cause for the changes in threshold but merely one expression of those processes which underlie the accompanying changes in threshold.

Though these voltage drifts resemble in many points those observed in peripheral nerve and the spinal cord,[22] they are usually much larger and more prolonged. The negative drift in Fig. 13[2] for instance lasted about 20 sec., the ensuing positive drift between 5 and 10 minutes! Points of resemblance are: *a.* the sequence, *i.e.* negativity followed by positivity; *b.* the susceptibility to various conditions, such as temperature, poor local blood supply or poor general circulation, partial asphyxia. In our experiments on the cortex the depth of Dial-narcosis has been shown (Fig. 13) to be also of

great importance, though we are not in a position to exclude entirely some element of lesser ventilation, since the animal breathed spontaneously. Finally these voltage drifts are augmented with increase of the number of neurones discharged and with the number of times they are discharged. This resemblance is not surprising if one considers the great number of nerve fibers in the cortex.

But irrespective of whether the processes underlying these voltage drifts are the same in cortex, cord and nerve, their association with the change in threshold to repetitive stimulation is always the same; negativity is associated with increased responsiveness, positivity with decreased responsiveness. It is of interest to point out that while the manner of picking up these voltage drifts on the cortex (without injury) is entirely different from that of picking up the after-potentials in an excised peripheral nerve (from longitudinal and cut surface), both are explicable in terms of the membrane-theory, for in both negativity is associated with a decrease and positivity with an increase of the "membrane-potential" in cells or fibers that have been discharged; the essential condition being that one electrode is at the site of this discharge, the other at an unaffected site, be it a remote focus in the cortex or the killed end of a peripheral nerve.

We have so far discussed the neural activity evidence by electrical activity at the time and place in question and the slow voltage drifts indicative of threshold changes then and there. Both of these changes are concerned only with neurones already involved. It was because it was impossible to frame a plausible explanation for the facilitation encountered in the experiments with unequal pairs of stimulations, and because of the persistence of diminished electrical activity and extinction beyond the period of positive voltage drift that we were forced to seek for a third variable, which would affect the threshold not only of neurones that had been discharged, but also the threshold of any neurones in their vicinity, whether previously excited or not. It was obvious that this variable must involve changes in the "milieu" of the neurones in question and was, therefore, presumably a change in ionic constitution. Obviously an investigation of the pH of the cortex was in order and, when made, the pH was found to be significant.

(iii). The specificity of the axonal terminals of the myriads of nerve cells in a given area of the cortex is so great that the consequences of a change in pH of a given area, carrying with it the alteration of activity of cells not involved in the response to a given stimulus, cannot be seen as yet in detail. Antagonistic cortical foci lie often so close together as to be affected contemporaneously by a local pH change. For this reason it is difficult to see how changes in pH could be responsible for the rapid contrasting phenomena encountered in reciprocal innervation, which imply changes of opposite sign in neighbouring neurones. On the other hand the changes in threshold of a cell or cell-group associated with a slow voltage drift are discrete and limited to the cells discharged. In fact it is in terms of threshold changes of particular cells in the spinal cord that Gasser[21] has offered an explanation of re-

ciprocal inhibition. Thus it is possible to account for the inhibition en-
countered in reciprocal innervation in terms of a factor for extinction; it is
obviously impossible to account for extinction in terms of inhibition, since
extinction is a change in the system due to antecedent activity in that same
system and, therefore, does not require a relation with an antagonistic
system.

(iv). In conclusion we wish to mention briefly one more point. It is of
interest to note that whereas the motor responses to constant intermittent
electrical stimulation of a "motor" focus show typical "waves,"[6,24] the
spontaneous electrical activity of the cortex, without stimulation, does not
show corresponding fluctuations. One is, therefore, driven to regard the oc-
currence of these waves as a consequence of the stimulation itself, *i.e.* as
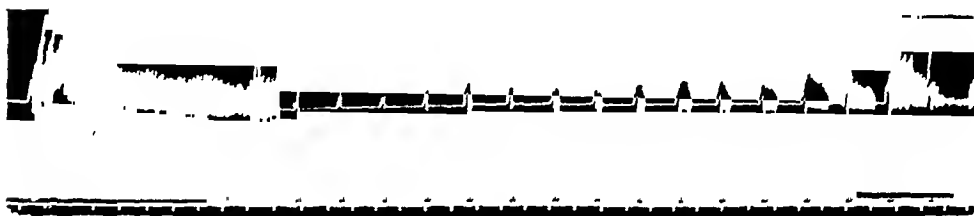
FIG. 30. Feb. 26, 1936. *Macaca mulatta.* Dial-narcosis. Continuous subthreshold
background stimulation at 5 per second of a "motor" focus for extension of wrist and in-
termittent repetitive stimulation (96 per sec.) at periods indicated by upper signal marker.
Time = 20 sec. The important thing to note is that the motor activity shown is *not* a motor
afterdischarge following the intermittent stimulations, but responses to the background
stimulation facilitated by the intermittent stimulations. The second indicates rapid and
slow changes in the responsiveness of the nervous systems involved. At the end of the
record a new slow cycle begins; the whole record represents, therefore, a little more than
one whole slow cycle of the order of 10 minutes' duration.

being due to factors for facilitation and extinction. It is possible to verify
this conclusion with the following experiment (see Fig. 30). One "motor"
focus is stimulated from two independent sources, one of which delivers a
background stimulation of 5 pulses per sec., which by themselves are just
subthreshold. The other source delivers once every 34 sec. for a period of 3
sec., stimulation at 96 short pulses per sec. which when applied for the first
time, and superimposed upon the background stimulation, results for 190
sec. in a series of responses to the latter stimulation. In Fig. 30 it will be seen
that the first responses of this series are very large, then gradually diminish,
then return to an intermediate size to end abruptly with a few large re-
sponses. During this period of responses to background stimulation the
intermittent stimulation was omitted, so as not to interfere with the facili-
tated motor responses to the background stimulation. After the cessation of
these responses the intermittent stimulation was reinstated and it will be
seen 1. that each of these stimulations results in a temporary reappearance
of motor responses to the background stimulation, 2. that these gradually
become larger and more persistent. At the end of the record an entire cycle

begins again. After all the foregoing the interpretation is not difficult. The first of the intermittent stimulations facilitates the response to the background stimulation in two ways: (i). by the relatively local changes of threshold and, (ii). by the more widely spread afterdischarge. The first of these runs a short course leading to extinction, producing a minimum of response at 40 sec. (positive drift at the site of stimulation), whereas the second runs a much longer course (typical of electrical afterdischarge in cortex and cord) to end abruptly in extinction (extinction due to involvement of remote cortical foci and spinal structures). The dissipation of this extinction is clearly exhibited in the latter two-thirds of the record. It is important to note that the prolonged facilitation which parallels an electrical afterdischarge cannot be repeated for many minutes, whereas the short-lived facilitation associated with negative voltage drift is repeatable within about half a minute after the end of the facilitated responses associated with electrical afterdischarge. This again confirms the finding mentioned sub D.4 (p. 343).

Figure 30 shows that regular intermittent repetitive stimulation of the cortex sets up slow rhythmical changes in its responsiveness and indicates that underlying the "waves" of cortical excitability are the more persistent factors for facilitation and extinction in parts of the CNS distant from the site of stimulation. These are, for facilitation, the electrical afterdischarge and its associated slow negative voltage drift (and probably alkalinity) and, for extinction, the diminution of electrical activity, its associated slow positive voltage drift and certainly acidity.

CONCLUSIONS

Facilitation and extinction are alterations induced in the response to test stimulation by antecedent stimulation. Underlying facilitation and extinction are changes in the activity and threshold of the parts of the CNS involved in the response to test stimulation. These changes, initiated by antecedent excitation, differ in kind and vary in intensity from time to time and from place to place.

For *facilitation* the factors are: (i). hyperactivity yielding increased summation; (ii). negative voltage drift associated with decrease of threshold in neurones previously involved; (iii). probably increase of pH (alkaline shift) decreasing the threshold of all neural structures in the region involved. For *extinction* the factors are: (i). hypoactivity resulting in less summation; (ii). positive voltage drift associated with increase of threshold in neurones previously involved; (iii). decrease of pH (acid shift), increasing the threshold of all neural structures in the region involved.

The effects of choice of the parameters of antecedent and test stimulation and of the interval between them upon latency, threshold and amplitude of response are explicable in terms of the modes of action of these factors.

The findings presented in this paper offer an approach to the problem of reciprocal innervation, for they compel now verified prediction of (i). the

conditions for apparent reversal of a cortical focus, and (ii). the changes in the knee-jerk following facilitating or extinguishing stimulation of a cortical focus for extension or flexion at the knee. Inhibition in reciprocal innervation is explicable in terms of the more discrete factors for extinction.

The "waves" of cortical excitability, previously described, are referable to the more remote and prolonged factors for facilitation and extinction.

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CONTROL BY THE CENTRAL NERVOUS SYSTEM OF RECTAL SMOOTH MUSCLE

ORTHELLO R. LANGWORTHY AND SEYMOUR J. ROSENBERG

Sub-department of Neurology, the Johns Hopkins University, Baltimore, and Veterans' Administration Hospital, Perry Point, Maryland

WE HAVE developed the theory that smooth muscle is controlled by reflex arcs involving nearly all portions of the central nervous system. Experimental and clinical observations have demonstrated its applicability to the muscle of the urinary bladder (Langworthy, Lewis, Dees and Hesser, 1936). The muscular wall of the different viscera is adapted to particular functional demands; this must influence the manner of its control by the brain and cord. The smooth muscle of the rectum has been chosen for extending this investigation, because it again is accessible for experimental work in mammals and man.

Stimulation of the cerebral motor cortex under favorable conditions modifies peristalsis in the gastro-intestinal tract (Watts, 1935; recent work has been summarized by Fulton, 1938, p. 479). Removal of the premotor cortex may produce violent peristalsis, giving rise to intussusception in monkeys. Patients with damage to the ventral surface of the brain stem are prone to develop hemorrhages and ulcers in the mucosa of the stomach and intestines. The observation that many epileptics have gastro-intestinal aurae has been correlated with the cerebral representation of these areas. Denny-Brown and Robertson (1935) have studied the relation of activity in the rectum to that of the anal sphincters, and the control of this mechanism both by the peripheral nervous plexus and by the spinal cord. Records were made on normal man, patients with cauda equina lesions and others with transection of the spinal cord. Contraction of the rectal musculature always led to relaxation of the internal and external anal sphincters. Since this relation exists, we have examined only the activity of the muscle of the rectal wall and disregarded the sphincters. Our observations have included experiments on 12 cats before and after removal of portions of the central nervous system, and records of activity of the rectal muscle in patients with hemiplegia and paraplegia. The experimental results are given here.

Our records were obtained by means of the apparatus shown in Fig. 1. A rubber balloon, approximately two inches long, which held 20 cc. of air before stretching began, was connected by means of thick walled rubber tubing to a U-shaped water manometer of 3 mm. bore. The distal end of the manometer was in turn attached to a tambour which recorded on a kymograph the transmitted changes of pressure within the balloon. The lubricated, uninflated balloon was inserted into the cat's rectum well above the sphincters. By means of a luer syringe 5 cc. increments of air were injected into the balloon through a T-tube. Air was prevented from escaping from the system by the use of a two-way valve. The height of the distal column of water was noted immediately before and after the instillation of air. The difference in height of this column of water was an indication of the relative changes of pressure occurring within the balloon. Although the pressure readings are not absolute values, the fact that we used the same manometer in all experiments renders the pressure changes directly comparable. Respiratory excursions were recorded by means of an inflated balloon fastened to the animal's chest and connected to a tambour.

Time was marked at 5 sec intervals Under deep ether narcosis, a tracheal tube was inserted and the cat's lungs were inflated periodically by means of a Harvard respirator A normal reading of rectal responses to progressive distention was made The carotid arteries were then tied, and the cat was decerebrated at the level of the superior colliculus After the bleeding was well controlled, another study of the rectal muscle was made In like manner readings were obtained following transections between the optic and acoustic colliculi below the vestibular nuclei, in the lower medulla, and spinal cord.

To determine the pressure changes due to the elastic properties of the balloon itself,
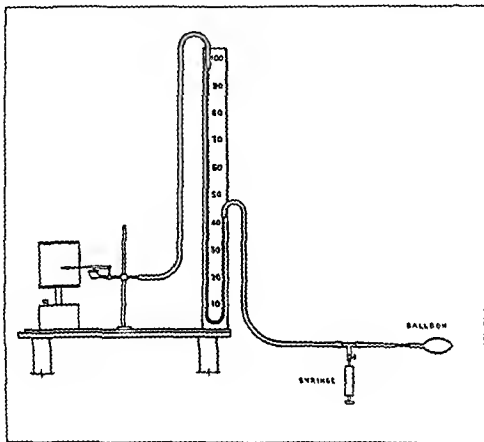
Fig 1

a graph was made by adding 5 cc increments of air to the balloon outside of the animal (Fig 2A) At low volumes there is an increasing resistance of the balloon to stretch, observed as greater changes in pressure for each increment of volume At greater volumes the resistance of the rubber to stretch diminishes until a point is reached where it is so negligible that no further changes in pressure appear to occur In our animal experiments no more than 11 increments of air were added to the balloon, so that only the first third of graph 2A need be used for comparison Readings were made in several animals 5 min after death (Fig 2B) It is known that smooth muscle lives for several hours after the death of an animal At the beginning of filling no marked increase of pressure was obtained on adding increments of air This is due in part to the fact that the balloon was not sufficiently inflated to produce stretch of the rectal wall Later the increments of pressure increased progressively as the constant volumes were added The record is similar to the first portion of distention in graph A Toward the end of filling rhythmic waves of rectal contractions were seen These show that the rectal muscle was still capable of contracting In these two graphs the endeavor was made to demonstrate resistance to distention of the rubber balloon alone and then of both the balloon and the relatively inert rectal wall These two are measured simultaneously in the experiments In the live animal a third important factor must be considered, the added influence of '
possible to procure an ideal type of preparation in which
in graph B Theoretically we should have waited for many
was quite dead. But then rigor mortis would have been a complicating factor On the other hand if the record had been made from a live cat with all the nerve trunks to the rectal wall cut, we would have measured abnormal responses either initially to "shock" or later to release from central control.

Since the results of all experiments were identical, only one typical case is presented. The reading in the normal anesthetized cat is given in graph A (Fig. 3). Pressure readings were noted immediately before and after the introduction of air so that such instillations are readily recognized. Six increments each of 5 cc. of air were introduced into the rectal balloon. Filling was then stopped because the pressure had reached the limit which could be measured by our apparatus. No stretch responses were elicited from the rectal muscle by the sudden distention of the balloon. A few rhythmical waves of muscle contraction were seen after the addition of the second increment of air. The smaller waves were oscillations produced by respiratory activity. The rectal wall showed more resistance to distention than in the dead animal (Fig. 2B).

ELASTICITY OF BALLOON

A

RECORD FIVE MIN. AFTER DEATH OF CAT

B

FIG. 2

The brain stem was then transected just above the level of the optic colliculi, and the anesthetic was stopped. A second record was made which is shown in graph B (Fig. 3). In this case nine increments of air were introduced. Superimposed upon the small respiratory waves, larger waves of contraction of the rectal muscle were visible. These were seen following the addition of the first three increments of air. They then diminished to start again with even greater amplitude as the rectum became distended. After the introduction of the seventh, eighth, and ninth quantities of air, a marked stretch response was elicited from the wall. Following the ninth instillation of air the pressure rose till the balloon was expelled. It appears from the graph that the rectal muscle was more irritable to stretch stimuli, and more waves of contraction occurred after section of the brain at this level than in the normal animal.

A second cut transected the brain stem between the optic and acoustic colliculi. Difficulty was encountered in inserting the balloon because of the resistance offered by the increased irritability of the rectal muscle. This record (graph C, Fig. 3) indicates that the rectum could not accommodate more than one increment of 5 cc. of air. The rectal wall contracted strongly in response to this stretch, producing two sharp, peaked waves of contraction. The pressure then fell, but slowly rose again and the balloon was expelled. It may be assumed that transection at this level resulted in a marked increase in tone of the rectal muscle which became hyperirritable to stretch. Diarrhea was observed in the animal at this phase of the experiments, and

may be interpreted as an increase in tone and irritability of the muscle in other portions of the gastrointestinal tract. The fourth record, shown in graph D, was made after the brain stem was transected in the upper part of the medulla below the vestibular nuclei. Ten increments of air were introduced into the balloon before the maximum pressure limit of our apparatus was reached. It will be recalled that only six increments could be instilled in the normal cat. The rectal wall was no longer hyperirritable. No rectal contractions and no responses to stretch were observed. Since the cut was made above the respiratory center, the respiratory phases were quite normal.

NORMAL

TRANSECTION ABOVE OPTIC COLLICULI

A

B

ABOVE ACOUSTIC COLLICULI   UPPER MEDULLA

LOWER MEDULLA

C

D
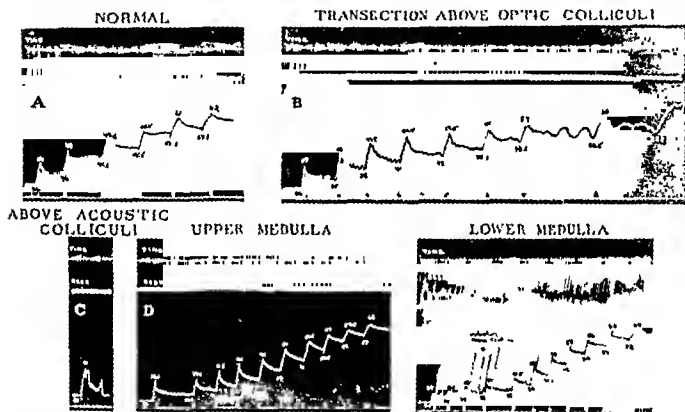
Fig. 3

The fifth record shown in this series (graph E, Fig. 3) was made after a transection through the lower portion of the medulla. Eleven increments of air could be introduced into the balloon. Again no stretch responses or rhythmic contractions were noted. The short spikes of pressure rise were due to spasmodic contractions of the striated muscle of the abdominal wall. At this level the cut was below the respiratory center; respiratory exchange was only made possible through automatic inflation of the lungs. The record of breathing is irregular because the diaphragm and intercostal muscles contracted strongly when they were stretched passively.

Other studies were made after section of the spinal cord in the thoracic region. These were quite similar to the records shown in graphs D and E.

DISCUSSION

Some years ago we demonstrated that tone in the smooth muscle of the urinary bladder was controlled by reflex arcs involving the midbrain (Lang-

Since the results of all experiments were identical, only one typical case is presented. The reading in the normal anesthetized cat is given in graph A (Fig. 3). Pressure readings were noted immediately before and after the introduction of air so that such instillations are readily recognized. Six increments each of 5 cc. of air were introduced into the rectal balloon. Filling was then stopped because the pressure had reached the limit which could be measured by our apparatus. No stretch responses were elicited from the rectal muscle by the sudden distention of the balloon. A few rhythmical waves of muscle contraction were seen after the addition of the second increment of air. The smaller waves were oscillations produced by respiratory activity. The rectal wall showed more resistance to distention than in the dead animal (Fig. 2B).

ELASTICITY OF BALLOON

FIG. 2

The brain stem was then transected just above the level of the optic colliculi, and the anesthetic was stopped. A second record was made which is shown in graph B (Fig. 3). In this case nine increments of air were introduced. Superimposed upon the small respiratory waves, larger waves of contraction of the rectal muscle were visible. These were seen following the addition of the first three increments of air. They then diminished to start again with even greater amplitude as the rectum became distended. After the introduction of the seventh, eighth, and ninth quantities of air, a marked stretch response was elicited from the wall. Following the ninth instillation of air the pressure rose till the balloon was expelled. It appears from the graph that the rectal muscle was more irritable to stretch stimuli, and more waves of contraction occurred after section of the brain at this level than in the normal animal.

A second cut transected the brain stem between the optic and acoustic colliculi. Difficulty was encountered in inserting the balloon because of the resistance offered by the increased irritability of the rectal muscle. This record (graph C, Fig. 3) indicates that the rectum could not accommodate more than one increment of 5 cc. of air. The rectal wall contracted strongly in response to this stretch, producing two sharp, peaked waves of contraction. The pressure then fell, but slowly rose again and the balloon was expelled. It may be assumed that transection at this level resulted in a marked increase in tone of the rectal muscle which became hyperirritable to stretch. Diarrhea was observed in the animal at this phase of the experiments, and

This number of the *Journal*
is dedicated
with respect and affection
to
Charles Scott Sherrington, O.M.
who introduced the concept of
The Synapse,
as well as the word itself,
into the literature of neurophysiology

PREFATORY NOTE

Symposium on the Synapse

THE PAPERS contained in the September number of the *Journal of Neurophysiology formed the subject matter of a Symposium on the Synapse held* at Toronto, April 29, 1939, during the Annual Meeting of the American Physiological Society.

THE EDITORS

AXONS AS SAMPLES OF NERVOUS TISSUE*

HERBERT S GASSER

Laboratories of The Rockefeller Institute for Medical Research, New York

THE FIRST question that would naturally be asked about the synapse is What is the nature of the material coming in contact at its borders? The answer to the question must come from a direct study of the synaptic region of the neuron, and for that reason it is a difficult one to obtain As an introduction to the problem, attention has been directed to properties of the axon, because of the expectation that the events which take place in those parts of the neuron entering into the synapse may resemble, qualitatively at least, events taking place in other parts of the neuron If it could be shown that the expectation has a foundation in fact, to the extent to which it holds, axon physiology could be transferred directly to synapse physiology

As is well-known, axons are not all alike This fact in itself is helpful for our present purpose, as samples of different kinds of nervous tissue are presented for review The common features among the characteristics of these samples may be taken to give an indication of the qualities that are shared generally by nervous structures, and the mode of variation of the characteristics may be taken to give an indication of the directions in which differences are to be anticipated

I want to mention with the greatest possible brevity the properties of the action in nerve fibers that appear to have application to synaptic conduction The illustrations which will be cited are typical for the three kinds of nerve fibers, A, B, and C The designation A refers to the somatic myelinated fibers, B to the autonomic myelinated fibers, that is, the group originally described by Bishop and Heinbecker as B_2, and C to the unmyelinated fibers

Action in all fibers starts with a spike The only difference between one type of fiber and another in this regard is in the duration (Fig 1) The A spike stands at one end of the range with a duration of 0 4 msec. and the C spike at the other end with a duration of a little over 2 msec

The spike is followed by an after-potential much smaller in size and much greater in duration After-potentials vary as to form, size, and duration, depending upon the kind of fiber (Fig 2) The complete sequence is a negative after-potential followed by a positive potential It is found clearly developed in A and C fibers In B fibers the negative after-potential is vestigial in single responses, and it appears only after certain forms of activity and after special experimental procedures When records are prepared at low amplification, as in Fig 2, so that both the spike and the after-potential are visible in the same tracing, the variation in the size of the positive after-potential

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at once strikes the eye. The positive after-potential is readily visible in the
B fibers and somewhat less so in the C fibers, while in the A fibers the con-
figuration that appears so clearly at higher amplification is all but indistin-
guishable.

Spikes are generally considered to be the message carriers; that is, some
agent, physical or chemical, directly under the control of the spike process
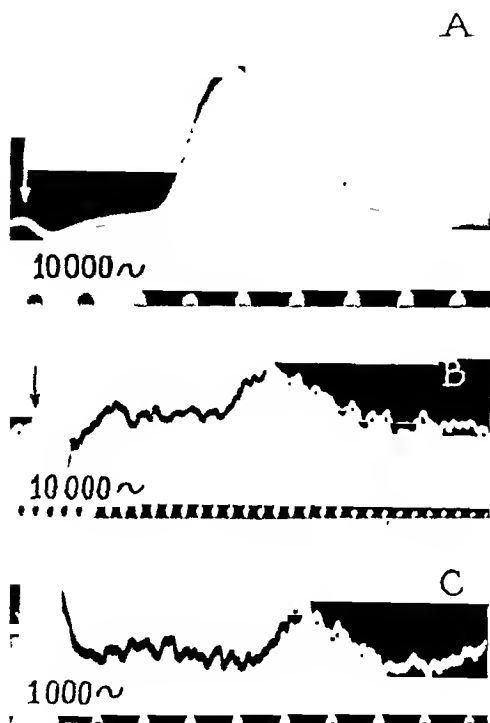
Fig. 1. Spikes of A, B and C fibers; A,
potential from a single large fiber of a dorsal
spinal root of the cat; B, from the cervical
sympathetic nerve of the rabbit, threshold
response, possibly not a single fiber (spike
25 μv.); C, from the splenic nerve of the cat,
threshold response (spike 20 μv.). The fine
oscillations are occasioned by the noise level.
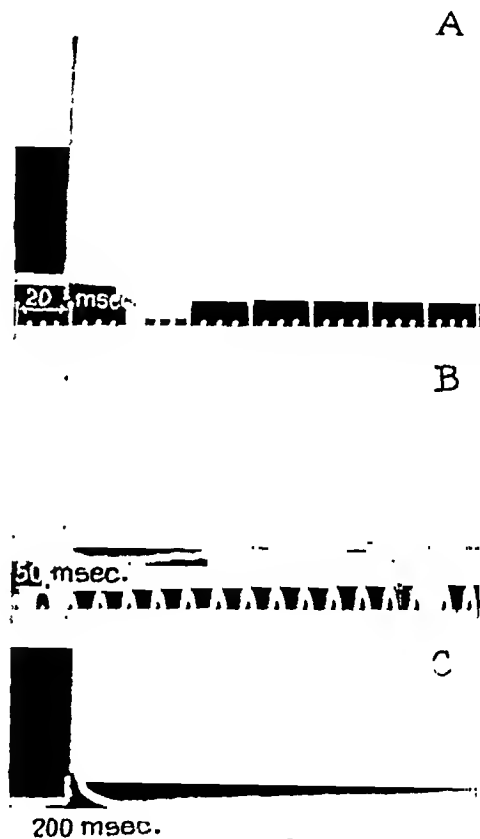
Fig. 2. Action potentials of A, B and C
fibers (cat): A, from the phrenic nerve; B and
C from hypogastric nerves.

is held responsible for the mediation of transmission. The view that spikes
serve this function is well founded, but other views have not thereby been
excluded. The contemporary literature contains arguments for impulse
initiation by slow potentials as well.[1]

One point is certain with respect to the after-potentials; they influence
the level of excitability. During the negative after-potential the fibers are
supernormal, and during the positive potential they are subnormal (Fig. 3).

Furthermore, when the configuration of the after-potential is altered, as it easily can be in a completely reversible manner by a wide variety of conditions or of states of activity, the excitability curve is altered in parallel with it. The same parallelism that holds between excitability and after-potential form for the various states of a single type of fiber, also obtains for the variation of the after-potential as it appears in the different types. The course
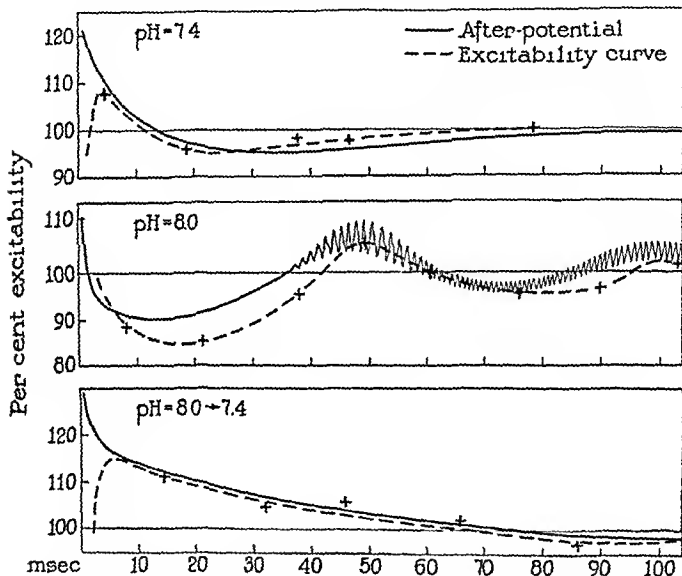
FIG 3 Relation between the excitability curves and the after-potentials in A fibers (phrenic nerve of the cat) Lehmann[8] (1937) The three parts show from above downward the normal condition, alkalinity resulting from removal of CO_2 from the atmosphere, and an early stage following restoration of CO_2 before the normal steady state is reached The ordinates give the reciprocal of the threshold for excitation in percentage of the resting threshold

of the excitability curves following a single response is plotted for the three types of fibers in Fig. 4. The curve for the C fibers[6] resembles that for the A fibers but for the fact that it is much more drawn out in time. As would be expected, the B fiber curve differs from both of them. In keeping with the absence of negative after-potential, there is no early supernormal period.

When a nerve is tetanized, the positive after-potential following the last spike in the train is larger than one following an isolated spike. The manner in which the growth takes place in A fibers is shown in Fig. 5, and an analo-

gous process occurs in the other types. If the tetanization is severe enough, the positive after-potential at the end of the tetanus, which corresponds to the one seen after a single response, is followed by a second positive potential. Unlike the first, the second potential increases in duration as well as size as the length of the tetanus and particularly the frequency of the tetanus are increased. The end result is the same in all fibers (Fig. 6)—an after-potential yielding records characterized by an initial sharp notch followed by a long
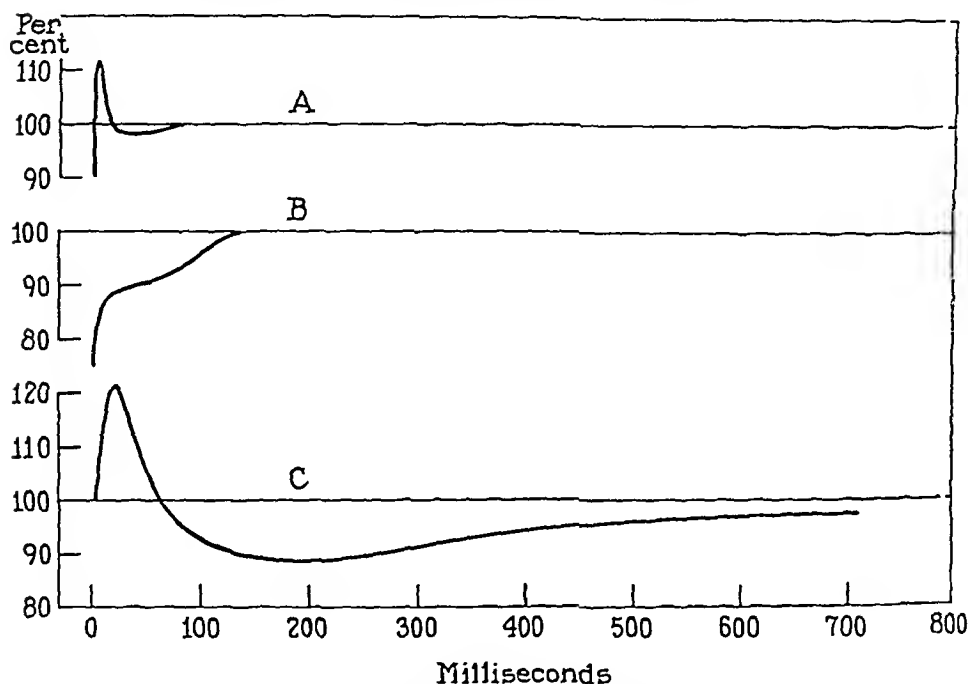
FIG. 4. Excitability curves of A, B and C fibers shown for comparison on the same time scale.

shallow trough. But for the time scale and the size of the potential in comparison with the height of the spike, the after-potential following a tetanus of C fibers resembles greatly that recorded after a tetanus of A fibers.

The excitability of a nerve following a tetanus is exactly as would be predicted from the after-potentials: the longer the duration and the higher the frequency of the tetanus, the greater and more prolonged the ensuing subnormality. Some of the early stages in the development of this subnormality are shown in Fig. 7. Among other things, the curves show that the supernormal period is a phenomenon restricted to very mild activity and that supernormality following the cessation of the tetani responsible for message transmission could hardly occur.
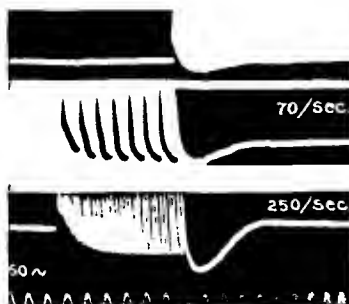
FIG. 5. Increase in the positive after-potential produced by a short tetanus (A fibers, phrenic nerve of the cat, 37°C., 5 per cent CO_2 in O_2). The records start with the negative after-potential. The spikes would extend far above the tops of the records. Changes in the negative after-potential are also to be noted.
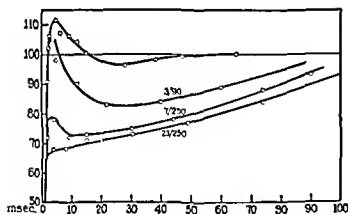
FIG. 7. Recovery of the sapbenous nerve in situ, decerebrated cat. The ordinates give the reciprocal of the threshold strength of stimulation in percentage of the resting threshold; the abscissae, the time after the end of the conditioning excitation. In the course of the experiment conditioning was changed from a single action to a tetanus. 3/90 means conditioning by 3 shocks at 90 per sec. (Gasser and Grundfest[4] 1936).
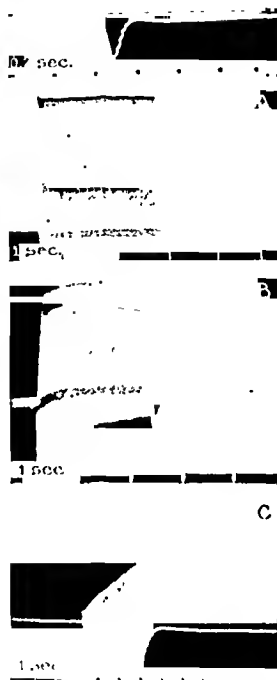
FIG. 6. Tetani of A, B and C fibers: A (lower), from the phrenic nerve of the cat; B and C, from the hypogastric nerve of the cat. The tops of the A and C spikes are at the tops of the records, the tops of the B spikes at the tops of the heavy white lines. The form of the A after-potential can best be seen in the upper record taken with higher amplification and with a faster sweep than in the record below it.

So much for the axon. Now, do any analogous phenomena occur at the dendritic end of the neuron? Numerous bits of information indicate that they do, but it would be impossible to summarize them within the compass
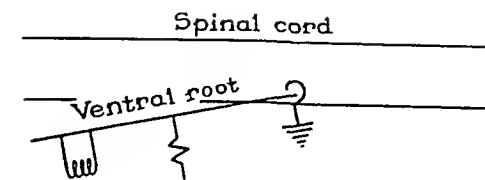
FIG. 8. Records obtained with the leads shown in the diagram when a single volley was backfired into the spinal cord of a cat through a motor root. (Dial narcosis). The lower record is at approximately 100 times the amplification of the upper. Control observations showed that the potential contributed by the spinal cord was not caused by a spread of the stimulating current to central structures but, as held by Eccles and Pritchard, was evoked by the antidromic volley itself.

FIG. 9. Leads as in Fig. 8. The record shows the potential evoked by an antidromic tetanus. (Dial narcosis.)

FIG. 10. Leads as in Fig. 8, except that both electrodes were on the root, the central electrode about 2 mm. from the cord. (Dial narcosis.) The upper record was produced by a single antidromic volley. The second record was produced by a single volley entering the cord through the dorsal root of the same segment. The potential was occasioned chiefly by the motor reflex discharge. The remaining records show the conditioning effect of the antidromic volley upon the size of the reflex. Arrows indicate the moment of stimulation of the dorsal root.

of the present discussion. Only one point will be brought forward, that is, subnormality in motor neurons. That motor neurons have a subnormal period similar to that of the axon first became apparent in considerations involving the interpretation of the silent period following a reflex discharge (Gasser,[3] p. 199). The arguments adduced in support of the association of the two events can now be passed over, however, because new and more direct evidence has become available from the experiments of Eccles and Pritchard,[2] in which a study was made of the effects of backfiring an antidromic volley of impulses into the spinal cord by way of a motor root.

Figure 8 was prepared in the course of repetition of one of Eccles and Pritchard's experiments. The first sacral motor root at its exit from the spinal cord was severed and stimulating electrodes were placed near the cut end. A single volley was backfired into the cord and the action potential led off from the side of the root and from the cord adjacent to the point of emergence of the root. The diphasic spike obtained at low amplification helps in the clarification of the potential picture obtained at high amplification. The latter has the appearance of a monophasic action potential of an A fiber (spike upward) as observed at high amplification (Fig. 5). Evidently, as concluded by Eccles and Pritchard, the form of the action potential of the intramedullary portion of the neuron is being revealed. If the cord is asphyxiated, the potential rapidly disappears and there is left only the after-potential of the root fibers. Under the conditions of the experiment, the centrally contributed portion of the potential so dominates the contribution from the root that in the algebraic summation of the two in the records, the configuration of the centrally produced potential is not obscured.

The duration and form of the central positive potential evoked by an antidromic volley are the same as the duration and form of the positive after-potential in a single action of A fibers. After a tetanus the similarity between the two potentials still holds. When a tetanus is backfired into the cord, the central positivity is increased and prolonged and the potential develops a two-part contour (Fig. 9) in keeping with the general pattern that holds for the after-potentials following tetani in all nerve fibers. The distinguishing feature in positive after-potentials is the first positive notch. In the motor neuron potential the notch corresponds to the one which is characteristic of A fibers.

That the locus of production of the central positive potential includes that part of the neuron in which the motor impulses are set up follows from the fact shown by Eccles and Pritchard that a motor reflex discharge is conditioned throughout the period during which the positive potential following a backfired volley persists. Records from a repetition of the backfiring conditioning experiment are shown in Fig. 10. They make it clearly evident that throughout the positive potential the excitability of the motor neurons is subnormal. A full-sized reflex is obtained only when the positive period is cleared.

The positive potential in the motor neurons not only has the duration of the positive after-potential in motor axons, but it is attended by the expected subnormality. There is indeed no reason for not calling it the positive after-potential of the neurons. The interpretation of the negative part of the central potential, on the other hand, is not so clear. In the experiment pictured in Fig. 10 there was nothing resembling supernormality; nor have we seen supernormality in other experiments. Recovery takes place along a continuously rising curve, just as it does in the internuncial neurons,[7] without separation of the refractory period and the subnormal period by a transient period of low thresholds. One may question whether there is any negative after-potential included in the recorded negativity. If there is, there must be an additional factor controlling excitability which has not yet been resolved. Quite possibly also under other conditions the finding may be different, as Eccles and Pritchard have described low thresholds during the negative period.

The peripheral endings of afferent fibers, which in a morphological sense are dendritic, provide another place where the conditioning effect of an antidromic volley can be tested. Here, too, there is a subnormal period. The number of impulses set up by a controlled tap on the skin is reduced as

20 msec
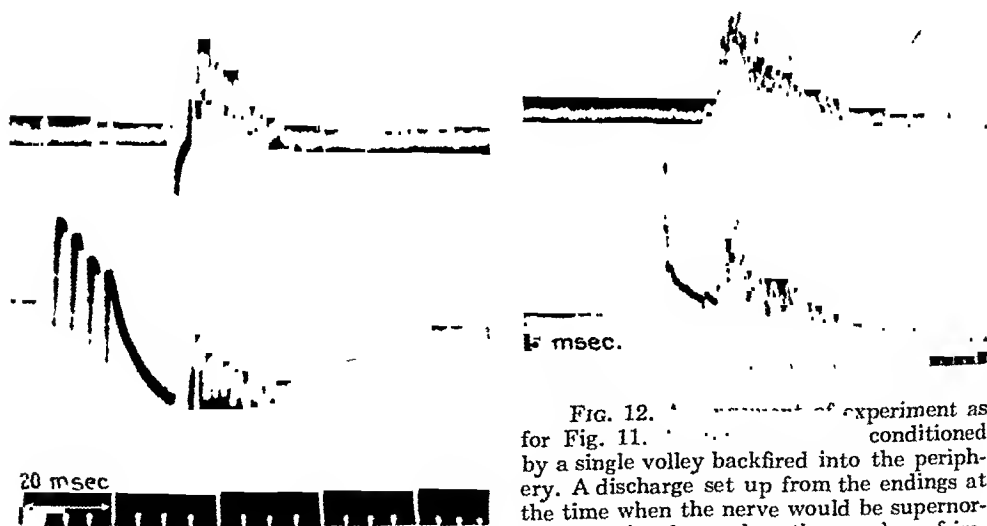
FIG. 11. Monophasic lead from the central end of a branch of the saphenous nerve of the cat. A gentle tap was applied to the skin of the leg with a device prepared by Toennies from a loud-speaker cone, and the discharge of impulses set up by the sensory endings recorded from the nerve (upper record). The discharges were reproducible as to size. In the lower record the size of the discharge has been conditioned by four volleys backfired into the periphery from a stimulating electrode on the nerve distal to the leads. The record shows four negative after potentials and the ensuing positive after-potential upon which is written the action potential of the conditioned discharge from the endings.

⊢ msec.

FIG. 12. A ... experiment as for Fig. 11. ... conditioned by a single volley backfired into the periphery. A discharge set up from the endings at the time when the nerve would be supernormal contains fewer than the number of impulses in the normal control.

long after the arrival of a backfired volley as the positive after-potential lasts in the nerve (Fig. 11). The probability, therefore, is strong that a positive after-potential is present in the endings. Supernormality, however, is absent, as it was seen to be in the motor neurons (Fig. 12).

Subnormal excitability following single spikes and trains of spikes is characteristic of all kinds of axons and all parts of the neuron. It is also found in all parts of the central nervous system, only it is there called inhibition. The significance of subnormality in the interpretation of inhibition makes the subnormal period, first described in nerve fibers by Graham,[5] one of the most important features of nervous activity which the axon has to present to the understanding of the synapse. The axon can also contribute to the understanding of facilitation, but the mechanism involved belongs in the group of phenomena which take place below the threshold of excitation, and facilitation, therefore, falls within the province of the next speaker.

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THE INITIATION OF IMPULSES IN AXONS

JOSEPH ERLANGER

Physiological Department, Washington University School of Medicine, St. Louis

IT IS QUITE generally believed that, to quote from a current textbook (Bard, 1938, p. 6), "where there are synapses conduction of excitation takes on certain characteristics that are not found elsewhere, *e.g.*, in nerve trunks composed of axons"; and that "there is some reason for attributing the peculiarities of central conduction to the synapse." These assertions, of course, hark back to Sherrington. How fundamental are the differences between nerve fiber and synapse conduction? I propose to devote my time to a consideration of that question, rather than limit my remarks exactly to the title as announced. I shall single out for consideration a few of the asserted differences with which my laboratory has had some experience, namely, latency, one-way transmission, repetition, temporal summation or facilitation and transmission of the action potential across a nonconducting gap.
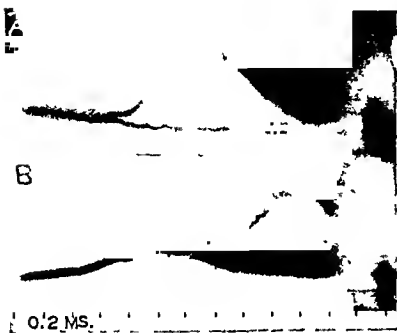
FIG. 1. Configuration changes in an axon spike at the locus of anode polarization.

A. The normal (unpolarized) diphasic axon spike on its bent base line.

B. *The higher spike* is the same axon spike, polarized anodally to the limit still permitting of conduction. The notches on the ascending limb (in this case somewhat atypical in spacings) are determined by loci of greatest susceptibility (nodes of Ranvier) of the fiber to anode polarization. *The lower spike* is the same spike after block at the first notch. The starts of the two spikes (blocked and unblocked) are superimposed. Block is indicated by the change from diphasicity to monophasicity of response. The lag at the node, due to the developing block, is of the order of 0.6 msec. *Time* is indicated in 0.2 msec. intervals.

To take up latency first, the action potential ceases to pass an anode block in a nerve fiber when the impulse is delayed there for an interval that is slightly longer than the time to maximum of the action potential. In the case reproduced as Fig. 1, for example, the block develops when the action potential lag amounts to about 0.6 msec. It seems safe to infer on the basis of observations on the relation of the duration of the action potential to conduction rate (Blair and Erlanger, 1933), that the time to maximum of the spikes in fibers of the sizes that ultimately reach the frog's neuromuscular synapse ranges between 0.3 and 3.0 msec. These figures about encompass the range of neuromuscular delays one finds in the literature. Granting block delay as the main factor determining latency, it follows that about the whole of the spike potential is needed for transmission at the neuromuscular synapse.

To pass to the next "peculiarity" of the synapse, namely, one-way trans-

mission, it has long been known (see Bishop and Erlanger, 1926) that the direction of conduction in nerve can be controlled by means of polarization. An impulse that can just pass when it progresses through a stretch of nerve successive parts of which are normal, anodally and cathodally polarized, and normal again, may be blocked when the succession is reversed, that is, from normal to cathodal, anodal and normal (Bishop and Erlanger, 1926). Bar-

FIG. 2. The effect of continuous anode polarization on the repetitive response of a fiber elicited at the cathode of a rectangular constant current. (During this experiment the ability of the fiber to repeat was diminishing progressively; this gradual change was not the result of the increase in the strength of the anode polarization.)

A. Repetition elicited in response to the rectangular current alone.

B–E. Prior to each of the records the continuous anode polarization was increased in strength. The added rectangular current starts at M in each case. *Time* for all records is linear and may be estimated by the distance from M to the end of record in E, which subtends 72 msec. The record also demonstrates facilitation by blocked nerve impulses; fortuitously the spikes often fail to reach the distal lead at first, as evidenced by their monophasicity; but the longer each of the records runs the more frequently is the spike conducted to the distal lead.

ron and Matthews (1939) believe that in the cord the direction of conduction through the synapse may not be fixed. If it is not, changes in a spatial sequence of excitabilities, such as can be effected in fibers by polarization, might very well determine the direction of readier conduction.

As for repetition, it has been known since the time of Pflüger that a nerve may respond repetitively during polarization with a constant current. The bursts of repetition thus started in a fiber as the cathode of a rectangular current can in some measure be controlled by varying the degree of steady

anodal polarization obtaining at the time the rectangular current is started (Erlanger and Blair, 1936), as may be seen in Fig. 2. Bronk (Bronk, Larrabee and Brink, 1938) undoubtedly will refer to experiments performed in his laboratory which have shown that alteration of the Ca and K content of peripheral axons may cause them to fire off just as does a motor nerve cell. Any other method of reducing accommodation should tend to make the nerve response repetitive.

We have yet to consider temporal summation and the transmission of impulses across a nonresponding gap. The rest of my time I shall devote to these topics.

To demonstrate temporal summation of subthreshold action potentials in a nerve fiber we have proceeded as follows (Blair and Erlanger, 1936). By continuous polarization of the small phalangeal nerve with the anode at the proximal lead, as indicated in Fig. 4, the impulse traveling along a particular fiber is blocked anodally at the lead. Electrical blocks develop at nodes of Ranvier, and as the polarizing current is increased, the first node to block would be the one most directly in the path of the current, and then *successively the less favorably* placed nodes. Figure 3 illustrates typically how the picture changes as the strength of the polarizing current is increased. The action potential first increases in height, then a notch develops on the ascending limb and at the critical polarization strength the part of the spike above the notch disappears. The action potential, if diphasic, becomes monophasic (see
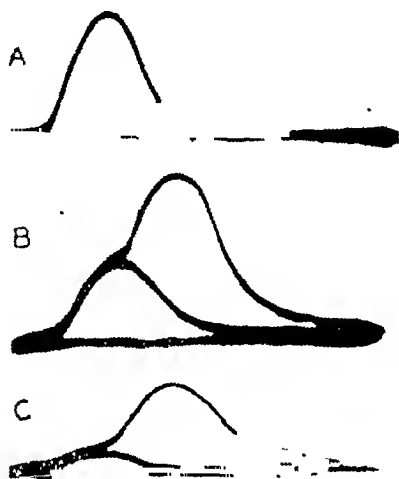
FIG. 3. Typical changes in the configuration of a monophasic axon spike produced by anode polarization at the proximal recording lead.

A. The normal spike.

B. The spike under anode polarization just strong enough to block at the most accessible node. Spontaneous variations in the fiber's excitability cause the block to fluctuate between one that is just not complete (the notched spike) and one that is just complete (the unnotched spike).

C. Further increase in the polarizing current to the next critical strength converts the unnotched spike of B into the notched spike of C, and when the latter is again blocked it records as the unnotched spike of C. The *time* is linear and may be estimated from the time to maximum of *A*, which is 0.37 msec.

Fig. 1 and 4). With further increase in polarization this process repeats itself at the next node removed, and so on.

Now if, when the first stage has been attained (that is, block at the most accessible node), and while it is being maintained by steady polarization (as in B 1–3, Fig. 4), a second action potential is made to follow the first, and blocked one, within a certain time range (here the interval is 3 msec.) the second action potential may be conducted through the block, as shown

here by the restoration of height and of the second phase. The first spike, though blocked, has so conditioned the blocked node (d) that a second spike is conducted through. This facilitation is maximal when the interval between the first and the second spikes lies somewhere between 2 and 4 msec., i.e., when most of the refractoriness following the first response has passed off, and declines slowly with further increase in the interval, facilitation becoming inappreciable when the interval reaches 80 to 100 msec. The spike lasts only about 1 msec.
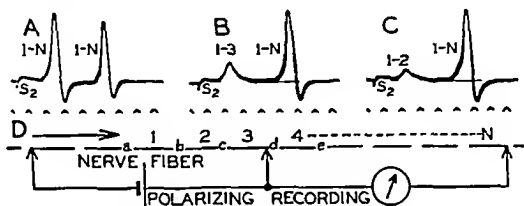
FIG. 4. Demonstration of facilitation in a nerve fiber extending the length of an internodal segment (possibly two) ahead of an impulse blocked by anode polarization. Judging by the results of the experiment, the electrode that served as anode of the "polarizing" circuit and proximal lead of the "recording" circuit occupied the indicated position relative to the nodes (a, b, c, etc.) and the internodes (1, 2, 3, etc.) of the responding fiber. With anode polarization short of blocking strength the conditioning spike recorded as 1-N in A. At the first critical blocking strength, A, 1-N changed to B, 1-3. At the second (and greater) blocking strength, B, 1-3 changed to C, 1-2. The second spike, in each case following the 1st after an interval of about 3 msec., is conducted through both blocks, at d in B, and at d and c in C.

The *time* is in milliseconds.

These values fit perfectly into the curve of temporal summation made by Bremer and Homès in 1930 through observations on the neuromuscular synapse of the frog. The preparations they used were slightly curarized, to the extent that one nerve volley failed of transmission whereas a second elicited a contraction. Their curves of contraction height against interval between nerve action potentials are reproduced here as Fig. 5. As I have said, our data derived from blocked nerve fiber fit these curves perfectly.

I have described the case where one action potential suffices to so condition the block that a second will pass. But anode blocks can be produced that are overcome by any desired number of impinging spikes, the number needed depending upon the strength of the blocking current.

Now how far beyond the anode block does the unblocking action of the blocked impulse extend along a nerve fiber? At least the length of 1 internode and in all probability the length of 2, or an estimated distance of 2 to 3 mm. An experiment showing how this has been determined is illustrated in Fig. 4. The electrode that is acting as the common proximal lead and polarizing anode has by trial been put in such a position that the normal spike, shown as 1-N in A, is converted, by a degree of polarization, into
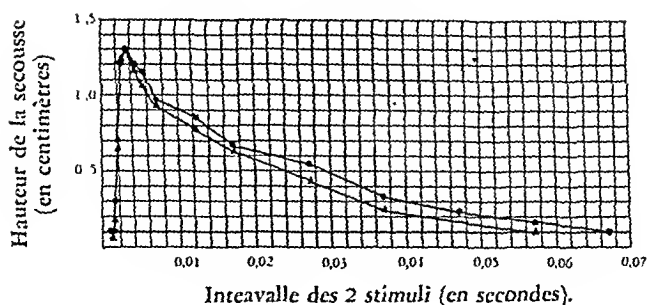
FIG. 5. Curves of temporal summation at the neuromuscular junction of the frog (from Bremer and Homès). The preparation was so curarized that the first of two successive nerve impulses failed to elicit a contraction. The height of the contraction is plotted against the interval between the two stimuli.

B(1–3) and this, by a slightly greater degree of polarization, into C(1–2). B is believed to picture anode block at node d, with restoration of conduction through d by facilitation, and C, 1–2, to picture the extension of block to node c, with restoration of conduction by facilitation not only through node d but through node c, also, giving C, 1–N. Here the conditioning influence of the action potential blocked at node c is extending from node c to node d certainly, and probably to node e.

Hodgkin (1937) also has shown, but by another method, that the excitability of nerve is raised for a considerable distance beyond a blocked nerve impulse.

It is interesting to compare temporal summation by blocked *action potentials*, with temporal summation by two *subthreshold electrical shocks* delivered in succession through the same electrodes. Previous observations (Erlanger and Blair, 1931) on multifiber preparations have shown in response to a subthreshold shock (see Fig. 6) a summation period lasting 0.2–0.6 msec. followed usually by a depression interval, a period of postcathodal depression, lasting 3–4 msec. Blair recently (1938) has plotted for the most irritable fiber of the phalangeal preparation the local changes in threshold following the delivery of a subthreshold shock. This was done with the fiber in three states, namely, normal, anodally polarized and cathodally polarized. Sample curves are shown in Fig. 7. Under all three conditions there is, of course, a summation
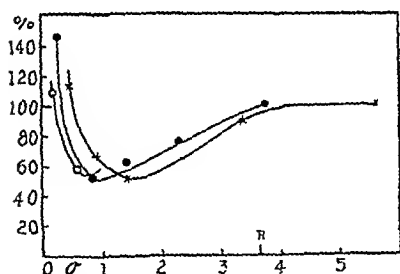
FIG. 6. Three curves showing latent addition or summation followed by postcathodal depression in in a nerve. The conditioning shock is 53, 79 and 97 per cent of threshold for the curves indicated by the circles, dots and crosses, respectively. The testing shocks, applied at the intervals shown on the abscissa, had a fixed strength such that they would under all conditions be within the submaximal range, and the height of the resulting multifiber spikes is plotted as ordinates in per cent of the spike height elicited by the testing shocks when delivered alone. The latent addition period increases from 0.2 and to 0.5 msec., approximately, as the strength of the conditioning shock increases. The subsequent period of postcathodal depression lasts 3–4 msec.

period. But in this experiment only the cathodally polarized fiber exhibits the previously described period of postcathodal depression Inconstancy of the period of postcathodal depression had been noted previously, but the factors responsible for its variability remained unknown. The state of polarization of the preparation evidently is one of them.

Another new observation, made by Blair, and also by Gasser (1938) independently, is that the period of postcathodal depression, when present, passes after 3–4 msec. into another and a long lasting, though low, summation period.
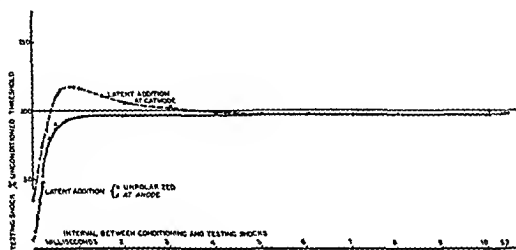
FIG 7 Curves (exhibited and described by Blair, 1938), but not previously published) showing the effect of a subthreshold shock upon the threshold of a nerve fiber. The conditioning shocks were 94, 64 and 90 per cent of the thresholds of the fiber when normal, anodally polarized and cathodally polarized, respectively. The strength of the testing shock in per cent of the threshold of the unconditioned nerve is plotted as ordinates against the interval between the conditioning and testing shocks. The cathodally polarized fiber (squares) shows latent addition, postcathodal depression and, after 4 msec, a second period of enhanced excitability (lowered threshold) lasting beyond 50 msec. The normal (circles) and the anodally polarized (triangles) fiber does not pass through a period of postcathodal depression and remains demonstrably hyperexcitable beyond 50 msec

In the case of the normal and of the anodally polarized fiber, as has been said, Blair found no period of postcathodal depression. Instead the latent addition or summation period is continued into a long drawn out period of slowly rising threshold. Therefore under all of the conditions that Blair dealt with, the threshold of the fiber, altered by a subthreshold shock, is below the normal level after 4 msec. have elapsed. After 50 msec. the threshold in all cases still is 1 per cent below normal.

Gasser, using multifiber, responses, has covered much of this ground independently (1938), has defined some of the conditions which will yield the long drawn out period of enhanced excitability following a subthreshold shock, has recorded associated potentials and has shown that this period of enhancement accounts for the recruitment of the fibers of a nerve when it is stimulated repetitively at rates as slow even as 20 per sec. with shocks of a constant strength initially below the threshold. Just as a considerable

number of *action potentials* may have to impinge upon a blocked locus in a nerve fiber to overcome an anode block, so, as Gasser has shown, the delivery of a number of *subthreshold shocks* through the same electrodes may be needed to cause a fiber to fire off.

Gasser's and Blair's experiments taken together then show that a subthreshold action potential would be expected to induce two periods of heightened excitability if the tissue it impinged upon were in a state resembling that produced by cathodal polarization, an immediate rise with a duration of the order of 0.4 msec., and a later rise beginning after about 3–4 msec. and lasting longer than 50 msec.; while Blair's experiments show, in addition, that if the state of the tissue resembled that induced by anodal polarization there would be but one period of summation, very intense for about 0.4 msec., but still appreciable after a lapse of more than 50 msec.

But quite as important from the standpoint of this symposium is the obvious similarity of the excitability reactions of muscle beyond a synapse to a subthreshold nerve action potential, of nerve beyond a block likewise to a subthreshold action potential, and of nerve to a succession of subthreshold electrical shocks. We may include here also Lorente's (1935) demonstration of the additive effects of induction shocks and action potentials on the excitability of motoneurons. The common denominator obviously is electrical.

Spatial summation, also, can be demonstrated in nerve fibers. Lorente, for example, has shown (1938) that subthreshold shocks may sum to produce a response when they are delivered to points as widely separated as 12–15 mm. And spatial summation of a blocked action potential and a shock has been plotted by Hodgkin (1937).

Synaptic transmission involves stimulation across a nonresponding gap and we thus far have concerned ourselves with continuous conduction, though, to be sure, with conduction made continuous through a process of facilitation. Next we wish to present evidence, now in press,[1] indicating that the nerve impulse actually can *stimulate* across a *nonconducting gap in* a fiber. Figure 8 is illustrative of the experiments demonstrating the phenomenon. Here the proximal lead electrode, P, is the *cathode* of a polarizing current and the two polarizing electrodes, the cathode and the anode, are separated by a distance which is slightly less than the assumed internodal distance. A separation of 1 mm. has been found to be satisfactory. This pair of electrodes is shifted on the nerve, and the current through them varied, until the desired result, to be described, is obtained. The evidence indicates that the electrodes then occupy a position with respect to two adjacent nodes (c and d) such as is depicted in the diagram. In the absence of polari-

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[1] This paper has since appeared (Blair and Erlanger, 1939). In the same issue of the *American Journal of Physiology* are two papers, one by Rosenblueth and Luco (1939) and one by Luco and Rosenblueth (1939), in which results are recorded which are regarded as "incompatible with the electrical theory of neuromuscular transmission." It may be stated here that the results recorded in them in no way qualify the conclusions reached in either the present paper or in the first paper mentioned above.

zation the diphasic action potential 1 is recorded. As polarization is increased 1 changes through all gradations into 2. At a critical polarization strength the successive records then change without any regular sequence into the configurations 2 to 6. Spike 2 may change into 3 by breaking at the notch indicated by the arrow; there are no pictures intermediate in configuration between these two; the change is all-or-none. Spike 3 changes through all gradations into 5. Finally, 5 changes into 6, and this change, like that from
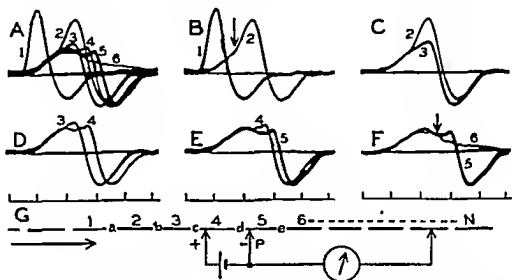
Fig 8 Six records of the spike of an axon, selected from a large number, showing that the action potential of a fiber can be made to stimulate the fiber across the length of one, possibly of two, nonresponding internodal segments The surmised positions of the polarizing and recording electrodes with respect to the nodes of the fiber whose spike is being recorded are shown in G The normal spike, 1, is diphasic At the critical polarization strength the spike's configuration, due to the fiber's spontaneously changing excitability, changes without any regular sequence as indicated by 2 to 6 There are no configurations intermediate between 2 and 3 or between 5 and 6 There is every transition between 1 and 2 as the polarization is increased to the critical level, and between 3 and 5 at the critical polarization

C is believed to picture anode block at node c with stimulation at node d by current determined by the activity of internode 3, and F is believed to picture cathode block at node d with failure of further conduction of the impulse

2 to 3, is without intermediate stages; it is all-or-none, the break taking place at the arrow.

The change from 2 to 3 without intermediate stages can only mean that the anode is blocking at node c. *The record* (3), *however, remains diphasic;* this must mean that despite the block the impulse continues to reach the distal lead. The potential determined by the response of internode 3 must be restimulating the fiber at node d.

Likewise, as I have said, the change from record 5 to record 6 occurs without any intermediate stages. The picture here indicates that we are dealing with cathode block; it must be at node d. This block, however, behaves as a block should; it stops the propagation of the impulse to the distal lead, witness the disappearance of diphasicity. If this interpretation of these pictures be correct, and we can see no flaw in it, we are dealing here with restimulation of the fiber at node e by potential associated with the activity

of internode 3. Two internodes, 4 and 5, have failed to fire off and yet the fiber is restimulated beyond.

Now, by way of discussion, let me recall the alternative hypotheses of neuromuscular transmission put forward by Dale, Feldberg and Vogt (1936). Either the nerve impulse directly excites the muscle cell but cannot reach its threshold unless the muscle is sensitized by a chemical substance produced *at the nerve ending*, or else a transmitter substance released *at the nerve ending* directly excites the muscle.

Transmission in a blocked nerve fiber fits neither of these alternatives. The stretch across which the axon potential can exert its effect, an internode or two, considering the time available, precludes the intervention of any process dependent upon substances released *at the nerve ending;* of any process dependent upon molecular continuity, such as secretion. The influence exerted by the potential difference upon excitability beyond the nonconducting gap alone can account for the results. It must be that if the spike itself fails to "detonate" the fiber beyond the gap, it so alters the fiber's composition there through electrochemical means that the threshold, lowered practically immediately, requires 100 msec. to return to normal. In any event, if an inactive stretch of fiber over 1 mm. in length does not stand in the way of electrical transmission of the impulse, is it reasonable to maintain that the discontinuity at a synapse will stop such transmission?

If I have succeeded in showing that there is a close correlation temporally between (i) the subthreshold effect of a shock applied to a fiber, (ii) the subthreshold effect of an action potential beyond a block at a node and (iii) the subthreshold effect of an action potential blocked at the neuromuscular junction by curari I will have succeeded in covering my assignment. If incidentally I have presented evidence indicating that an electrical mechanism can account for synaptic transmission whatever else may be involved, my defense is that it is a question of sufficient relevancy to the subject of the symposium to have justified the liberty I have taken.

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SYNAPTIC MECHANISMS IN SYMPATHETIC GANGLIA

DETLEV W. BRONK

Eldridge Reeves Johnson Research Foundation and the Institute of Neurology,
University of Pennsylvania, Philadelphia

I

THE INTEGRATED activity of the nervous system is determined by the fixed structural relations of the constituent neurons and by the variable influence of one neuron upon another. Through the excitation of nerve cells by contiguous cells the rhythmic processes in the individual units are compounded into the pattern of activity which determines the functions of the nervous system at any moment. This intercellular relationship is established by the processes at the synaptic junctions. The nature of those processes constitutes the problem of synaptic transmission. The problem has three principal aspects.

It is at first necessary to consider the nature of the events in the presynaptic neuron, for they constitute the primary agents of excitation. Direct measurement has shown during recent years that the characteristic activity of nerve fibers is the transmission of trains of rhythmically recurring impulses (Adrian, 1932; Bronk, 1938). As these impulses reach the synapse there is accordingly a sequence of reversible changes in the terminal portions of the fiber. The character of this alteration is not invariable, for the properties of the fiber vary with the frequency of action and antecedent activity. The character and effects of an impulse in the termination of any fiber will therefore vary from time to time.

Inasmuch as a nerve impulse is a reversible alteration of the physicochemical structure of the nerve, it follows that the immediate environment of the presynaptic termination is modified by each impulse. How the properties of the synaptic region are altered by the trains of impulses which arrive at the synapse is accordingly a *second* major problem in the study of synaptic mechanisms. That can be investigated by chemical studies of the fluids which pass through this region or by measuring as an index the altered properties of the adjacent nervous tissue. By the altered environment the properties of the postsynaptic cell are modified, and under suitable conditions one or more impulses are discharged over its axon. A *third* aspect of the problem is therefore a determination of the nature of these changes in the secondary neuron which are developed by presynaptic impulses.

For the experimental study of these several phases of the problem of synaptic transmission a sympathetic ganglion is a favorable preparation. The sequence of impulses which naturally come to the ganglion from the central nervous system can readily be determined. Alternatively, the pathways from the centers may be interrupted and electrically initiated impulses sent into the ganglion over a variable number of preganglionic fibers at con-

trolled frequencies. Impulses directly excite the ganglion cells without the intervention of internuncial neurons, and their response can be measured in terms of the postganglionic impulses which they discharge. A ganglion has, furthermore, a readily isolated blood supply which enables the experimenter to perfuse with solutions of determined composition, and thus it is possible to vary the environment of the cells or to study the chemical changes produced by nerve impulses.

The results of investigations on a sympathetic ganglion cannot, of course, be applied directly to an interpretation of synaptic mechanisms in the central nervous system. There is, however, a wide-spread belief that we shall be able to learn much about the general problem from these relatively simple structures. They have accordingly been extensively employed.

In the brief space of this discussion it would not be possible to review adequately the extensive literature that has grown out of those studies. Fortunately that has been made unnecessary by the recent reviews of Brown (1937), Dale (1937), Eccles (1936, 1937, 1939), Rosenblueth (1937) and of Bronk and Brink (1939). I propose, therefore, to give a brief summary of certain results of a series of investigations which have been carried out in our laboratories during the past few years and which deal with aspects of the problem little considered by other workers. Our primary purpose has been to analyze the factors that modify the activity of a ganglion cell and which thus regulate the fluctuating patterns of nervous activity. We have given particular attention to the influence of chemical agents, to the synaptic effects of trains of impulses corresponding to those which naturally course over the presynaptic pathways, and to the prolonged modification of cellular properties developed by antecedent activity. Because of the characteristic asynchronous action of groups of neurons we have frequently found it necessary to record the events in a single unit. For the information which I have at my disposal I am indebted to my colleagues D. Y. Solandt, S. S. Tower, R. J. Pumphrey, J. B. Gaylor, Frank Brink, Jr., and especially to M. G. Larrabee whose experimental skill is responsible for much of this work.

II

The cells of a sympathetic ganglion are naturally excited by trains of impulses coming to the synapses from the central nervous system over varying numbers of fibers and at frequencies which wax and wane as the activity of the centers fluctuates under the influence of afferent stimuli (Adrian, Bronk and Phillips, 1932; Bronk, Ferguson, Margaria and Solandt, 1936). We shall see that these variations in the number of preganglionic fibers which are conducting impulses and in the frequency of their action are important factors in determining the degree of synaptic excitation.

Inasmuch as the frequency of impulses in the postganglionic neurons grades the response of the effector organ, it is important to know whether the frequency of impulses discharged from the centers is modified by transmission across the synapses of the ganglion. This is a question that has been

frequently asked (Cannon, 1914; Querido, 1924; Veach and Pereira, 1925; Bishop and Heinbecker, 1932; Knoeffel and Davis, 1933; Brown, 1934; Bronk, Tower, Solandt and Larrabee 1938). In the earlier work an answer was sought by comparing the contractions of the nictitating membrane when the preganglionic and when the postganglionic nerves of the corresponding superior cervical ganglion were stimulated alternately, at various frequencies. Although there was some disagreement among the observers as to the interpretation of the results, most of them concluded from the indirect evidence that a ganglion does not modify the frequency of incident impulses. The most direct method of answering this question, and others pertaining to synaptic transmission, would be to record the trains of impulses coming over a single preganglionic fiber to a certain ganglion cell, while recording the discharge of impulses from that cell over its postganglionic fiber. Because this is well-nigh impossible the experimental procedure has been to

FIG. 1. Diagrammatic sketch of stellate ganglion of the cat showing the preganglionic nerves used for stimulation and the postganglionic nerve in which the cellular discharge is recorded.

stimulate the preganglionic trunk with electric shocks which thus initiate volleys of impulses in many preganglionic fibers and consequently in many postganglionic neurons.

In our own work we have usually employed the stellate ganglion of the cat,—a preparation which has many useful characteristics (Fig. 1). The first to the fifth thoracic roots as well as the preganglionic trunk are exposed and transected. It is then possible by electrically stimulating one or more of the several roots to send impulses into the ganglion over various pathways or over different numbers of fibers. The discharge from the ganglion cells, which is initiated by the preganglionic impulses, is recorded in the postganglionic fibers of the inferior cardiac nerve. The height of the spike potential recorded from many fibers is an index of the number of ganglion cells which are in action. The magnitude of the potential will, however, also vary because of changed properties of the postganglionic axons, due, for instance, to the persisting effects of previous impulses. The influence of such variables must therefore be carefully considered when the postganglionic action potential is used as a measure of variations in the activity of the ganglion cells.

The characteristic response of a group of these cells to a synchronized

preganglionic volley is a burst of impulses in the postganglionic nerve trunk (Fig. 2). The considerable width of the spike potential is due to the temporal dispersion of the individual axon spikes as they reach the recording electrodes. This dispersion is the result of differences in synaptic latencies and conduction times along the various fiber and synaptic pathways. That it is not due to a repetitive discharge of impulses from each ganglion cell is shown by records from single postganglionic fibers (Fig. 3), for these reveal a single sharp spike corresponding to a single impulse for each preganglionic stimulus (Bronk, Tower, Solandt and Larrabee, 1938). On the basis of such evidence one may say that a ganglion cell does not normally respond repetitively to a single preganglionic volley. From this we must conclude, as have Eccles (1935) and Lorente de Nó (1938), that the excitation developed by slowly-recurring volleys of presynaptic impulses does not persist at a supraliminal level after the discharge of an impulse from the cell. Synchronized volleys of impulses at frequencies not exceeding 20 per second consequently

FIG. 2. Oscillographic records of the postganglionic spike potential resulting from a preganglionic volley. Recorded at 6 and 30 mm. beyond the ganglion to show increased temporal dispersion. Arrows indicate shock artefacts. Time: 0.05 sec.

develop trains of impulses of a like frequency in each of the active postganglionic fibers.

Such a one-to-one correspondence between the frequency of synchronized impulses in presynaptic fibers and in postganglionic neurons does not justify the conclusion that a ganglion cell normally discharges impulses at
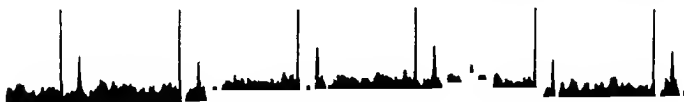
FIG. 3. Discharge of impulses in a single postganglionic fiber in response to preganglionic volleys. No repetitive discharge. Marker represents stimuli at 0.2 sec. intervals.

a frequency which corresponds to the frequency of action of a cell in a sympathetic center. The old and much debated question whether the rhythm of impulses from the central nervous system is modified in a ganglion cannot be answered by the use of electrically initiated volleys of synchronized impulses in many fibers. For one does not thereby initiate

frequently asked (Cannon, 1914; Querido, 1924; Veach and Pereira, 1925; Bishop and Heinbecker, 1932; Knoeffel and Davis, 1933; Brown, 1934; Bronk, Tower, Solandt and Larrabee 1938). In the earlier work an answer was sought by comparing the contractions of the nictitating membrane when the preganglionic and when the postganglionic nerves of the corresponding superior cervical ganglion were stimulated alternately, at various frequencies. Although there was some disagreement among the observers as to the interpretation of the results, most of them concluded from the indirect evidence that a ganglion does not modify the frequency of incident impulses. The most direct method of answering this question, and others pertaining to synaptic transmission, would be to record the trains of impulses coming over a single preganglionic fiber to a certain ganglion cell, while recording the discharge of impulses from that cell over its postganglionic fiber. Because this is well-nigh impossible the experimental procedure has been to

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the cell ove ~~....~~ ... ~~.. ...~~ ... the same fiber. Whether
a ganglion · ~~.....~~ erefore upon the number
of presynaptic fibers conducting impulses to it. This is well illustrated by
Fig. 4 which shows in the first record the postganglionic discharge developed
by stimulation of one root. The height of the spike potential is determined by
the number of ganglion cells that are active. In the second record, similar
volleys were sent into the ganglion over root A during repetitive stimulation
of root B. The increased responses to the volleys in root A show that certain
additional cells were activated by the summation of the effects produced by
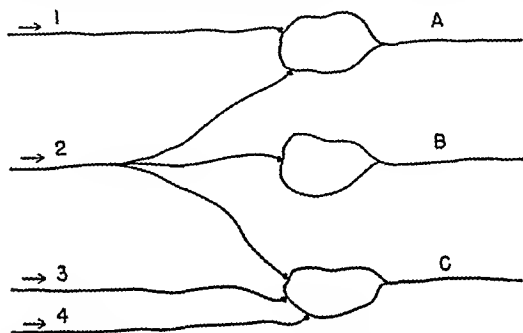
FIG. 5. Schema illustrating the control of alternative pathways in the nervous system.

the train of impulses in one set of endings with the effects developed by the
volleys in another group of fibers. This example of the well-known phenome-
non of spatial summation is cited here partly to emphasize the multiple
innervation of ganglion cells (see also Eccles, 1935a and b). It also illustrates
in a simple manner how the effect of a train of impulses on a group of nerve
cells is modified by the concurrent arrival at the same group of cells of a
train of impulses from another source. Whether in Fig. 5 an impulse arriving
over fiber 2 will excite cells A and C may depend upon the activity in fiber 1
and in fibers 3 and 4. By such means the paths of impulses in the nervous
system are undoubtedly being shifted from moment to moment with conse-
quent alterations in the pattern of activity.

The excitation of a ganglion cell may also depend upon the frequency
with which impulses arrive at one or more of the synapses. If the excitation
developed by a presynaptic impulse persists for some time, the subliminal
effects produced by each of a series of presynaptic volleys should ultimately
summate to a threshold level. This is indeed what happens. Figure 6 shows
the progressive increase in the heights of the postganglionic spike potentials
initiated by a train of uniform preganglionic volleys. Inasmuch as the

magnitude of the spike potential in the postganglionic nerve is a measure of the number of fibers which are conducting impulses, it will be seen
that there is a progressive recruitment of ganglion cells. How many additional units are brought into action during an extended period of repeated stimulation depends upon the number of cells that are at the
beginning of the train subliminally excited by the incoming impulses. The
degree of recruitment is accordingly greater when only a fraction of the
preganglionic fibers innervating a given group of cells is excited or when
the excitability of the ganglion cells is low. For the purposes of our discussion the significant fact is this. Trains of impulses such as those which
constitute the messages of the nervous system tend to recruit more and more
cells into action. Because of the decay of the excitatory state the number of
additional cells which are thus activated is a function of the frequency of the
presynaptic impulses. Inasmuch as the frequency of impulses is an important
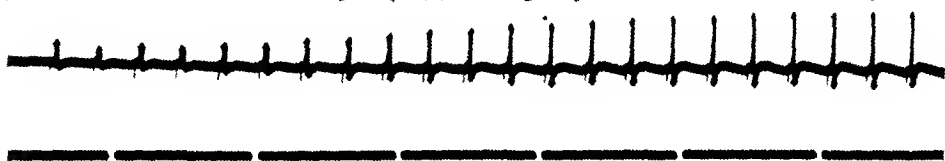
FIG. 6. Increase of postganglionic spike potentials during train of preganglionic volleys.
Shows progressive recruitment of ganglion cells. Time: 0.5 sec.

variable in the messages of the nervous system, it is interesting to find that
variations in frequency of presynaptic impulses modify not only the rhythm
of the postsynaptic cells but also determine the number of cells in action.
There will also be a selective response of certain neurons in the nervous
system to certain rhythms, for the cells with higher thresholds will come into
action later in a train, or respond only to higher frequencies.

IV

If the preganglionic nerve is stimulated at frequencies greater than about
20 per second, the successive volleys do not evoke progressively increasing
postganglionic action potentials. On the contrary, the spikes become smaller,
the rate of failure depending upon the frequency of stimulation (Bronk and
Pumphrey, 1935; Rosenblueth and Simeone, 1938). This may be due in part
to a progressive block of synapses by the rapidly recurring impulses. However, an examination of the discharge in individual fibers from single ganglion cells reveals another and significant reason for the decrease in height
of the spike potential in the postganglionic trunk. At these high frequencies
many of the cells discharge at rates which do not correspond to those of the
presynaptic volleys. Because the rhythm of discharge is different in the various cells there is a temporal dispersion of the postganglionic impulses with
a consequent decrease in the height of the synchronized spike potential.

We do not at the present time have much information concerning the factors that govern the frequency of this independent rhythm of a ganglion cell, but we may assume that a certain level of "excitatory state" is developed by any given number and frequency of impulses. This causes the cells to discharge at rates which are governed by the degree of excitation and by the characteristics of the individual units. The frequency of impulses necessary to produce these effects is greater than any we have observed coming from the sympathetic centers. But, the resulting discharge of impulses at frequencies not directly related to the frequency in any one presynaptic fiber and at unrelated rhythms in the several postganglionic units is similar to that of a ganglion in normal activity.
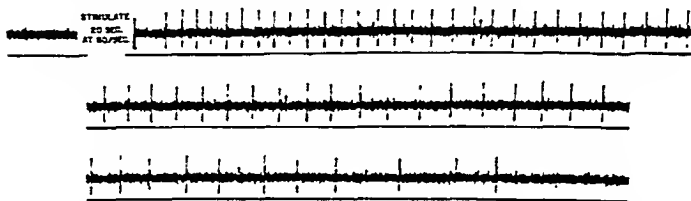
Fig. 7. Repetitive after-discharge from a ganglion cell following 20 sec. of preganglionic stimulation at 60 per sec. Continuous record. Last impulse 27 sec. after end of stimulation. Time: 0.5 sec. (Larrabee and Bronk, 1938)

Many cells which have been discharging impulses in response to a train of rapidly recurring preganglionic volleys (50 or more per sec.) continue in action for some time after the end of the preganglionic stimulation. Figure 7 is a record of such an after-discharge of impulses from a cell which continued its rhythmic activity for 27 sec. after the termination of the stimulus. Only gradually did the frequency decline, due presumably to the gradual decay of the "excitatory state." The duration of this after-discharge is graded by the frequency and duration of the previous excitation. Because it seldom appears following frequencies less than 50 per second it must be considered a clue to the nature of synaptic processes and not a phenomenon associated with a naturally activated ganglion. Whether the prolonged action is due to the persistence in the cellular environment of some agent of transmission or whether it is due to the continued liberation of some substance from the presynaptic terminations cannot be stated at the present time. The latter possibility is however reminiscent of Lorente de Nó's report (1938) of the prolonged liberation of acetylcholine following tetanic stimulation of a preganglionic nerve.

V

Even more enduring effects of synaptic excitation are revealed by the following type of experiment. A volley of impulses is sent into the ganglion,

and the height of the resulting postganglionic spike potential is recorded as a measure of the number of ganglion cells activated by the preganglionic stimulus. Similar volleys are then repeated at recurring intervals for a limited time. At various instants after the end of the repetitive stimulation the response of the ganglion cells is again tested with the same intensity of preganglionic stimulus. Such tests show that the number of ganglion cells activated by a certain number of preganglionic impulses is much increased by the intervening activity. The degree of this increase and the extent of time during which it can be observed are functions of the frequency and duration of the conditioning train of impulses. In a certain case represented in Fig. 8 the number of cells responding to the preganglionic volley was increased two-fold by a 5-second train of impulses, and only after more than
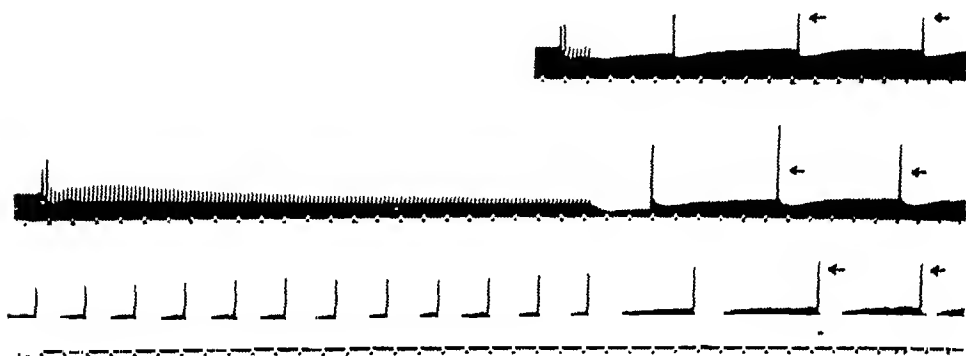
FIG. 8. Postganglionic responses to preganglionic volleys of constant size. Spike height is measure of number of cells responding. Three records showing increased response due to previous stimulation of varying frequency and duration. Time: 0.2 sec.

a minute was the postganglionic response again as small as it had been before the conditioning stimulation.

Although we assume that the conditions which make the preganglionic volleys more effective are developing progressively throughout the period of repetitive stimulation, there is during that time actually a decrease in the height of the successive postganglionic spike potentials. This need not, however, be considered as evidence against the recruitment of cells which did not respond to the initial volleys in the train. For as new cells come into action, those which have responded to the earlier volleys may drop out of action, or may respond only occasionally, because of an elevation of threshold produced by repetitive activity. The height of the postganglionic spike potential may be further reduced by the temporal dispersion described in a previous section. After the end of the repetitive stimulation, the elevation of threshold and the temporal dispersion must be assumed to disappear more rapidly than certain other changes within the ganglion. These latter changes are then revealed by an increase in the number of cells responding to a single preganglionic volley.

This prolonged increase in the response to presynaptic impulses might be due to a temporary modification of the properties of the presynaptic fibers, induced by the preceding impulses which they have conducted, There is certainly a persistent positive after-potential following activity, and with a concurrent increase in the axon spike potentials. It is possible that those characteristics of the impulse responsible for synaptic transmission would accordingly be more effective. The prolonged facilitation might, on the other hand, be due to a persistent change in the environment of the nerve cells, or to changes in the postsynaptic cells resulting from their previous activity. If antidromic stimulation modifies a ganglion cell as does stimulation through its synapses, we may rule out the last of these three factors. For
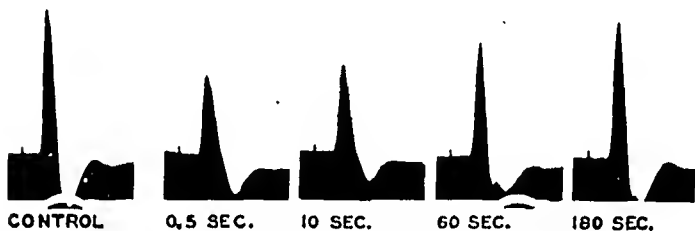
Fig. 9. Postganglionic action potentials developed by preganglionic volleys of constant magnitude. Before and at the various indicated intervals after antidromic stimulation for 1 min. at 20 per sec.

such excitation does not increase the number of cells which respond to a preganglionic stimulus.

On the contrary, the activity of a ganglion cell which is induced by stimulation of the postganglionic nerve decreases the capacity of the cell to respond to preganglionic impulses. Eccles (1937) has shown that the number of cells activated by a preganglionic volley is reduced for more than half a second after a previous antidromic volley. We confirm this and find that the postganglionic response may be decreased for some minutes following a train of antidromic impulses (Fig. 9).

Such a depression of the synaptic excitability of a cell must also occur as a result of activity initiated by presynaptic impulses. Under these circumstances, however, the reduced excitability of the cell does not cause a decreased postganglionic response, because it is masked by the increased effectiveness of preganglionic impulses. The train of impulses that initiates activity in the ganglion cells and thus causes a subsequent lowering of their excitability also develops conditions which increase the effectiveness of succeeding presynaptic impulses. If the time course of the two processes is not the same, the degree of facilitation may increase for a while following the end of the conditioning stimulus.

VI

Any analysis of the mechanisms responsible for the transient excitation produced by a single volley of presynaptic impulses, or for the more persistent modifications in the properties of a ganglion developed by a train of impulses, involves two considerations. One relates to the intrinsic changes in the preganglionic and postganglionic neurons which accompany their activity and continue for a time; the other concerns the influence on the ganglion cells of molecular and ionic alterations in their environment produced by the presynaptic impulses. In this latter category acetylcholine and potassium are of primary interest at the present time because both have been shown to be liberated in a sympathetic ganglion by preganglionic stimulation. (Feldberg and Gaddum, 1934; Vogt, 1936).

Fig. 10. Postganglionic spike potentials evoked by preganglionic volleys of constant magnitude. During upper signal 1.0 cc. of ACh was injected into perfusion fluid. Increased number of ganglion cells respond. Second record: 15 sec. later. Third record: 35 sec. later. Time: 0.2 sec. (Bronk, Tower, Solandt and Larrabee, 1938)

When a low concentration of acetylcholine in Ringer's fluid (Feldberg and Gaddum, 1934) circulates through a ganglion the number of cells responding to a volley of presynaptic impulses is increased. This is shown in Fig. 10, and the same effect is produced by increasing the concentration of potassium ions in the perfusion fluid. Whether these agents augment the response by increasing the excitability of the ganglion cells, or whether they modify some other factors in the synaptic mechanism, we do not know. In any event, their action is such as could explain the long persisting facilitation following a train of presynaptic impulses, provided it is shown that the acetylcholine and potassium outlast the period of excitation or continue to be liberated. We may also conclude that the liberation of acetylcholine by presynaptic impulses would have at least an important adjuvant action in synaptic transmission.

That there is a direct stimulating effect of acetylcholine on nerve cells is well known, for when it is perfused through the superior cervical ganglion the corresponding nictitating membrane contracts. (Feldberg and Vartiainen, 1935). This stimulating effect has the characteristics of a localized or specific action, for the ganglion cells respond to concentrations which do not excite preganglionic fibers or fibers running through the ganglion without synapse. This is well illustrated by an experiment represented in Fig. 11, wherein the activity of both the preganglionic and postganglionic nerves was recorded. Acetylcholine in a concentration of 100 μg. per cc. of perfusion fluid caused ·

a vigorous discharge of impulses from the ganglion cells, whereas even five times this concentration developed no impulses in the preganglionic fibers. That the presynaptic neurons were, however, accessible to exciting agents in the perfusion fluid is shown by their response to sodium citrate.

Because a chemical agent develops unrelated activity in the different neurons of a ganglion, its action is best studied by observing the discharge of impulses from a single cell. When this is done we find that acetylcholine initiates rhythmically recurring impulses which continue as long as the level of acetylcholine is maintained by the perfusion. There is no evidence
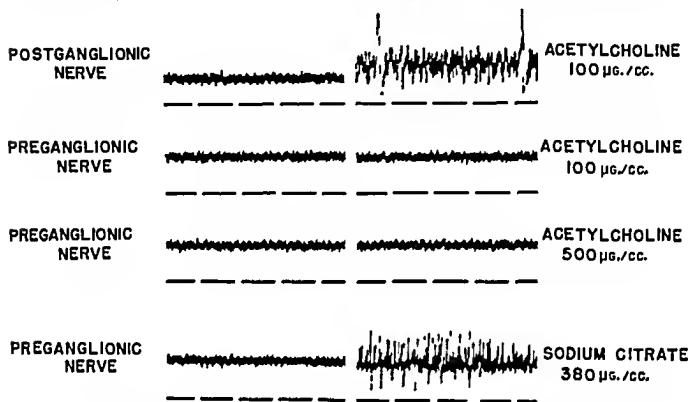
Fig. 11. Preganglionic and postganglionic responses to ACh and sodium citrate. Controls with Ringer's fluid in left hand column. Time: 0.1 sec.

of adaptation or failure for many minutes provided the concentration is below that which causes paralysis. The degree of this activity is graded by the concentration of the acetylcholine. Changes in the concentration modulate the frequency of discharge. This is shown in Fig. 12 which is the record of impulses from a cell the rhythm of which was increased from about 1 per sec. to 3 per sec. by raising the concentration of acetylcholine from 25 to 100 μg. per cc.

The effectiveness of acetylcholine and other stimulating agents varies, of course, from cell to cell. There is accordingly a wide range in the concentrations necessary to excite and in the frequency with which the cells discharge under the influence of the same concentration. Nor is the response of any one cell the same from moment to moment except under carefully controlled conditions. Variations in other chemical components of the environment and additional activity of the cell are two important factors that modify its rhythm.

A
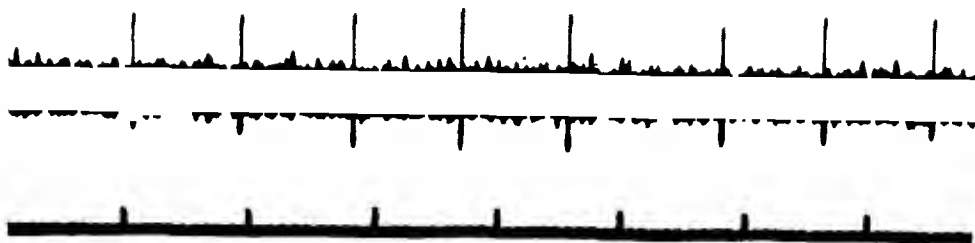
B

C
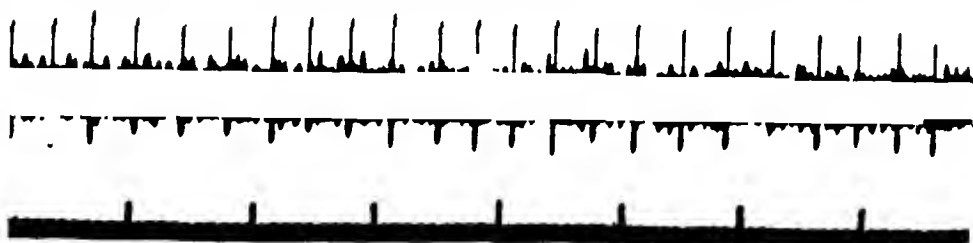
Fig. 12. Discharge from a cell in a ganglion perfused with A: 25μg.; B: 50μg. C: 100μg of ACh per cc. of Ringer's fluid. Time: 1 sec.

The activity of a cell excited by acetylcholine is for example modified by changes in the concentration of calcium or potassium in the environment. Such an effect is illustrated in Fig. 13. The second record in either column shows the impulses coming from a nerve cell in a ganglion perfused with normal Ringer's fluid containing 40 μg. of acetylcholine per cc. When the calcium concentration in the fluid was doubled the frequency of impulses discharged by the acetylcholine-activated cell was greatly reduced for several minutes. A similar reduction in the rhythm was produced by the alternative procedure of lowering the potassium. On the other hand, an increase in the concentration of potassium ions or a reduction of calcium augmented the rate of activity developed by a given amount of acetylcholine. These ex-
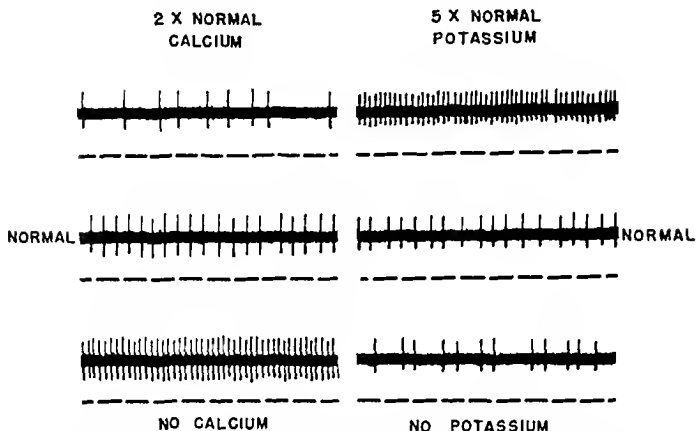
FIG. 13. Discharge of impulses from a single cell in a ganglion perfused with solutions containing 40μg of acetylcholine per cc. Middle records are with ACh in normal Ringer's fluid; others modified as indicated.

periments emphasize the general principle that the degree of activity of a nerve cell is determined by the combined effects of the various agents in the cell's environment.

VII

Another element of importance is oxygen. If the normal circulation of a ganglion is stopped or if the oxygen is removed from the perfusion fluid, the threshold of the cells for stimulation by acetylcholine or potassium is increased after a short time. Usually within the course of about ten minutes this becomes manifest as a decrease in the postganglionic discharge, due presumably to a reduction in the number of active cells and a decline in the rhythm of those which are still in action. After 30 to 60 minutes all response to the chemical agent has disappeared. No cells can then be activated even by high concentrations of potassium or acetylcholine.

During the time that the cells are losing their ability to respond to chemical excitants there is a progressive block of synaptic transmission. A gradual decrease in the height of the postganglionic spike potentials, initiated by preganglionic volleys of constant size, shows that fewer cells are being excited by the incoming impulses. Finally, the response to chemical stimuli and to presynaptic impulses is abolished at about the same time. The relative effects of asphyxia on the processes within the presynaptic terminations and on the postsynaptic cells are not known, but the parallel time course of

failure for chemical and synaptic excitation suggests that the irritability of the cell fails as soon as any part of the synaptic mechanism.

Schröeder (1907), Cannon and Burkett (1913) Bronk and Larrabee (1937) and Bargeton (1938) have pointed out that the failure of ganglionic transmission is partially reversible after somewhat more than an hour of complete anemia. Such a revival of excitation at a synapse is shown in Fig. 14. The arrest of circulation through a perfused ganglion had in that experiment produced the characteristic and progressive block in an increasing number of synaptic pathways. Transmission was finally abolished in all units after about 60 minutes and remained absent during the succeeding 6.5 hr.
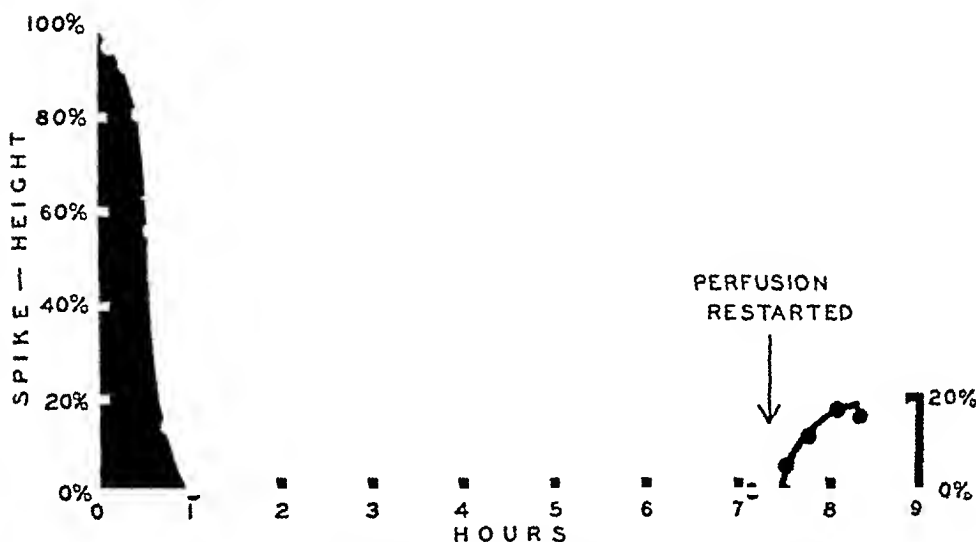
FIG. 14. Failure of ganglionic transmission during arrested circulation; followed by partial recovery.

Perfusion was then started again and within a brief interval 20 per cent of the cells were again responding to the preganglionic volleys. These experiments show that the properties of the presynaptic and postsynaptic units involved in the process of junctional transmission can be regained after being inoperative for more than six hours under asphyxia.

It has been widely held that transmission across synapses is readily blocked by asphyxia. We were therefore surprised to find that conduction over those preganglionic fibers which course uninterruptedly through the ganglion fails as soon as does transmission over the pathways containing synapses. The fact was established in the following manner (Larrabee, Gaylor and Bronk, 1939). At one minute intervals maximal shocks were applied to the preganglionic trunk while recording the resulting volleys in the inferior cardiac or cervical sympathetic nerves. From the latter nerve especially two distinct groups of impulses are recorded; the first comprises impulses in fibers which can be shown to pass continuously through the

ganglion, the later is the discharge from cells within the ganglion. The time course of failure for both of these is about the same. From this we infer that the processes involved in synaptic transmission are no more susceptible to lack of oxygen than are those responsible for conduction over certain axons.

The persistence of synaptic transmission in sympathetic ganglia throughout many minutes of asphyxia appears to be in marked contrast with the rapid failure of activity in the central nervous system. This is probably largely due to differences in the rate of metabolism of the nerve cells in the two locations (cf. Gerard, 1937). We must also bear in mind that the slow rate of failure of ganglionic transmission that has just been described pertains to synaptic pathways which are conducting at infrequent intervals. When they are caused to transmit trains of impulses at a frequency of five or ten a second their ability to conduct fails much sooner. Any comparison of the rate of failure of activity in the central nervous system and in sympathetic ganglia should undoubtedly be made relative to ganglia in continual activity. For that is the normal condition of many cells in the centers and in the ganglia as well.

VIII

This is another instance of the necessity for considering the effects of repetitive activity on synaptic processes when one is analyzing the mechanisms of this junctional transmission. The activity of a postsynaptic neuron is regulated not only by the rhythmic impulses in the presynaptic terminations, but also by the previous activity of the postsynaptic cell itself. Antecedent activity is accordingly an important factor in the determination of the fluctuating patterns of nervous action within an anatomically fixed system of neurons. The ultimate effects of a train of impulses in a neuron depend upon the irritability of the nerve cells which it innervates, and that is profoundly modified by previous events.

In a series of beautiful experiments Gasser and his colleagues (1937a, 1937b) have traced the time course of irritability changes in peripheral nerve following the conduction of a train of impulses, and have correlated the irritability cycle with the sequence of after-potentials. They thus find that an increased negativity of a region of a nerve is an index of increased irritability, whereas the irritability is decreased during the positive phase. The after-potentials are accordingly a valuable measure of the excitability of nerve.

By the use of various electrical leads from the ganglion or from the postganglionic nerve Eccles (1935a, b, and c) has similarly attempted to follow the altered properties of the ganglion cells which are induced by a previous impulse. Whether the variations in potential difference thus recorded actually relate to the surface of the cell body or whether they are a measure of the properties of the postsynaptic cell axons within the ganglion is still a debated question. But it is not improbable that the time course of the after-potentials in both portions of the neuron is the same. In

any event, Eccles finds that the ganglion cells are more readily excited by a preganglionic volley during the negative phase of the potential cycle following a previous volley, and fewer of the cells are excited during the positive phase. This correlation of the excitability of ganglion cells to presynaptic impulses with the after-potential cycle is then analogous to the correlation of after-potential cycles in peripheral axons with their electrical excitability. This analogy might indeed be taken by some as evidence favoring synaptic transmission by circulating currents.

Rosenblueth and Simeone (1938) point out that the decreased response of the postsynaptic cells following a previous volley of impulses may be due not to a decreased excitability of the cells but to a decrease in the efficacy of
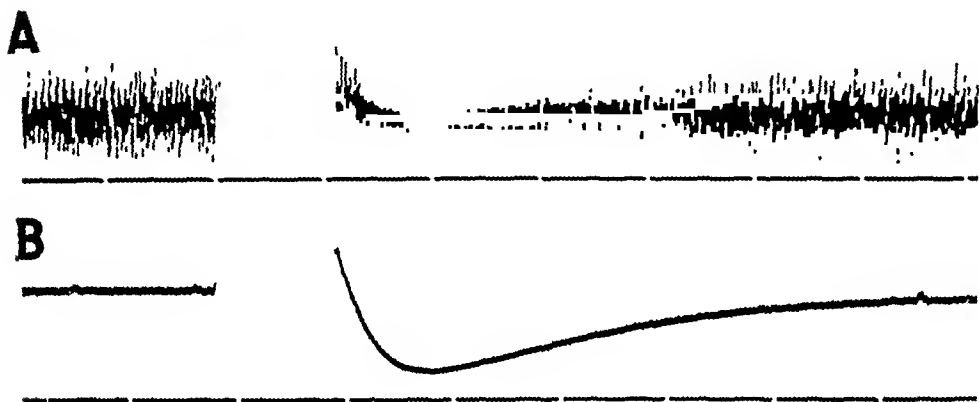
FIG. 15. Above: Discharge of impulses in postganglionic nerve during perfusion of ganglion with Ringer's fluid containing 100μg. ACh per cc. During break in record preganglionic volleys were delivered at rate of 40 per sec. Below: Ganglion potential (leads on ganglion and postganglionic nerve). Time: 0.5 sec.

the preganglionic test volley. They thus emphasize an important factor to which I have often referred in the present communication. It is certainly necessary to recognize as they do that activity in presynaptic fibers modifies their subsequent properties, and may therefore alter the effects produced at the synapse by succeeding preganglionic impulses. Because of such considerations and because in their own work they find a lack of correlation between the after-potentials and the responsiveness of the ganglion cells, Rosenblueth and Simeone question whether there is a general relation between ganglion potentials and synaptic excitability.

They furthermore raise the pertinent issue: "if nerve impulses do not act at synapses as electrical stimuli but excite the succeeding neurons by some other mechanism, then it need not be expected that the two after-potential phases should be attended by increased or decreased responsiveness. . . . For it is not known whether or not the threshold of the ganglion cells to acetylcholine varies during the after-potentials, or during the post-tetanic period. Such knowledge may provide a test for the (chemical) theory." This is an

important question which leads us back to a consideration of factors which modify the chemical excitability of ganglion cells.

We have already described how the response of the cells to a chemical agent such as acetylcholine is altered by the presence of other substances. The persistent influence of activity is now shown in Fig. 15A. At the left is the beginning of a record of the impulses discharged from many cells in a ganglion perfused with Ringer's fluid containing 100 μg. of acetylcholine per cc. We then sent volleys of impulses through the ganglion at the rate of 40 per sec. for half a second. This is shown as a break in the record, for the magnitude of the synchronized postganglionic spike potentials was sufficient to carry the electron beam off the face of the oscillograph. Immediately after the end of the train of impulse volleys there was a brief increase in the response of the ganglion cells to acetylcholine, and then an almost complete cessation of the discharge. Not for three seconds did the activity increase to what it had been before the cells were excited by the train of preganglionic impulses, Such experiments show that additional activity alters for some time the response of nerve cells to chemical agents.

Whether this sequence of changes in chemical excitability of the ganglion cells is related to a cycle of ganglionic after-potentials is answered by a comparison of Fig. 15A and B. The latter is a record of the ganglion potential which shows a brief initial negative phase followed by an increased positivity which lasts for some seconds. It is readily apparent that the increased discharge of impulses from the cells which has been described comes during the period when the ganglion is negative, relative to inactive tissue: the decreased activity during the period of positivity. There is accordingly a remarkable parallelism between the cycle of ganglionic after-potentials and the chemical excitability of ganglion cells. Inasmuch as the correlation between the sequence of after-potentials and the electrical irritability cycle of axons corresponds to that between ganglion potentials and the chemical excitability of ganglion cells, we can probably hope for no test here that will decide between the electrical and chemical theories of synaptic transmission.

It has been shown by Grundfest and Gasser (1938) and by workers in our laboratory that the magnitude and duration of the after-poentials of axons are determined by the frequency and duration of the preceding trains of impulses. The corresponding irritability changes are similarly regulated. Inasmuch as Rosenblueth and Simeone (1938) observe that the ganglionic after-potentials are likewise varied by the frequency and duration of the conditioning stimulus, we might expect to find that the frequency of discharge from chemically excited ganglion cells can be graded in a corresponding manner.

For such an analysis it is necessary to follow the activity of a single neuron. This has been done in experiments such as that represented in Fig. 16. In both A and B the upper record is of the ganglion potentials. The pronounced after-positivity is seen following the train of impulses sent in over the preganglionic trunk. How the magnitude of that potential can be graded

by the frequency of the conditioning volleys is strikingly illustrated by a comparison of A in which the stimulus was at the rate of 9 per sec. and B in which the rate was four times as great. The changed properties of the ganglion cells, which are revealed by these ganglion potentials, modify the cellular response to a chemical excitant such as acetylcholine. This is evident from the lower records of Fig. 16 A and B. A is the record of what appears to be the discharge from not more than two cells under the influence of a perfusion fluid containing 50 μg. of acetylcholine per cc. The activity ceases for somewhat less than two seconds during the peak of the positive after-
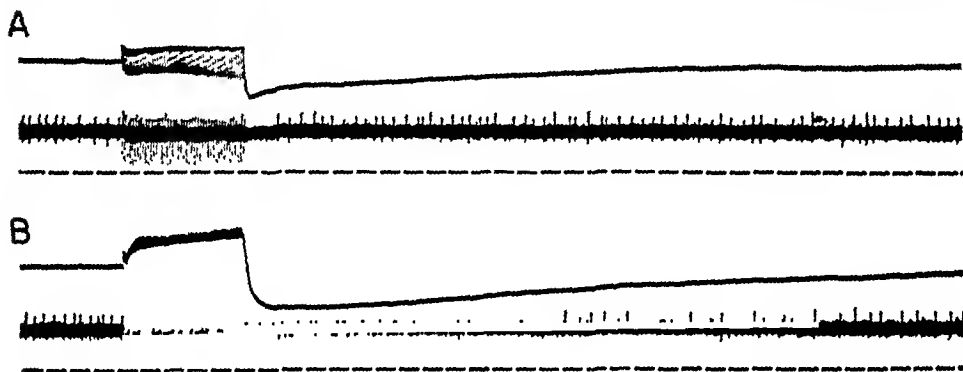
FIG. 16. Upper records in both A and B: Ganglion potentials developed by 5 sec. tetanus at rates of 9 per sec. in A and 36 per sec. in B. Lower records in both A and B: Discharge of impulses from one or two cells in the ganglion perfused with Ringer's fluid containing 50μg. ACh per cc. Inhibition of discharge during peak of positive after potential. Time: 1 sec.

potential. In B, on the other hand, the cell fails to respond to the acetyl-choline for 14 seconds after the period of rapid activity induced by the preganglionic volleys. And then only gradually does the rate of chemically induced discharge return to its original level, paralleling a decrease of the positive after-potential. Such a gradation of the frequency of response of a nerve cell to a specific chemical agent by variations in the frequency of impulses coming to the cell may be of great significance in regulating nervous action. For impulse frequency is a principal variable in nerve messages, as I have said before.

These changes in the chemical excitability of a ganglion cell must be ascribed to the previous activity of the postsynaptic neuron for the effects are the same whether induced by preganglionic or by antidromic stimulation. The lowered irritability is accordingly similar to the decreased response of ganglion cells to presynaptic impulses that follows a period of postganglionic activity. It will be recalled that in the latter case the lowered excitability of the cells can be observed only after the cells have been excited antidromically. For if they are conditioned by preganglionic impulses, the presynaptic terminations or the properties of the synaptic region are altered

in such a way that the effectiveness of the subsequent test volley is increased, and the lowered excitability of the ganglion cells is thereby masked.

We must now conclude that following the propagation of one or more impulses a ganglion cell is for some time less readily excited by chemical agents including acetylcholine; by presynaptic impulses; or—to draw an analogy from peripheral nerve—by electric currents. This raises the question of the relationship between these several modes of excitation, and that brings us to a consideration of the means by which preganglionic impulses excite adjoining ganglion cells.

I have no desire to defend either the acetylcholine hypothesis or the theory of excitation by circulating currents from the presynaptic terminations. On the other hand I do not wish to oppose them or to adopt a dualistic hypothesis. If it be necessary to do more at this time than describe the phenomena of transmission and relate them into a consistent scheme, I would argue ʿ   ʾ   ʾʾ ʿʾ, ʿʾ ···

If   by impulses at the terminations of preganglionic fibers, as abundant evidence shows, there is no doubt but that the properties of the ganglion cells will thereby be altered. In sufficient concentrations acetylcholine causes the ganglion cells to discharge impulses. In weaker concentrations it increases the ability of the cells to respond to presynaptic impulses. But similar effects are produced by potassium, and it is not improbable that an incident ·   ·   ·ʾ   ·ʾˀ   ·ʾ

tration of this and other ions at   ,

certainly the altered properties of the fiber terminations accompanying an impulse will give rise to a flow of current which will have some effect on the secondary neuron. Without attempting to evaluate the relative importance of any agent I would therefore urge the point of view that a presynaptic impulse modifies in many ways the environment of the contiguous cell body. The degree of excitation must then be determined by the summated effects of the various environmental factors—of those which tend to stimulate and of those which serve to depress.

It is furthermore necessary not to think of synaptic transmission as the development of one impulse by another. It is more probable, on the contrary, that a sequence of impulses in a number of fibers produces changes in the surroundings of a cell. The properties of the cell are thereby altered, and at a certain stage in the process an impulse is discharged.

Finally, the active agents in the synaptic mechanism should be considered as having variable characteristics. The presynaptic fibers conduct to the site of action, not isolated impulses, but trains of rhythmically recurring waves of activity of fluctuating frequency. Thus the properties of the fiber terminations and the characteristics of their impulses are continually changing, So, too, are the characteristics of the postsynaptic cells modified from moment to moment in accordance with their changing environment and their previous activity. By thus regulating the properties of the synaptic

junctions between nerve cells the rhythmic trains of impulses in the individual neurons create the changing patterns of nervous action.

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TRANSMISSION OF IMPULSES THROUGH CRANIAL MOTOR NUCLEI*

RAFAEL LORENTE DE NÓ

Laboratories of the Rockefeller Institute for Medical Research, New York City

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I. INTRODUCTION: PRIMARY CONSIDERATIONS

Differences between sympathetic ganglia and central nervous system

THE PRECEDING report of this Symposium written by Dr. Bronk deals with the transmission of impulses through sympathetic ganglia, *i.e.*, through synaptic relays of relatively simple anatomy. The present report is concerned with the transmission of impulses through organs of almost indescribable anatomical complexity,—the cranial motor nuclei and the pools of interneurons† that are connected with them. It is true that in many respects the neurons of the central nervous system are comparable to the sympathetic ganglion cells, and it is also true that the preganglionic fibers, after their entrance into the ganglion, divide and form arborizations comparable to those formed·by the afferent fibers of any pool of central neurons; but the similarity between the ganglia and the central nervous system does not go far beyond these points. Among others, the following difference is fundamental. All the fibers articulated with sympathetic neurons belong to a single tract (the preganglionic trunk) and consequently may be activated simultaneously by a suitable single stimulus, for example, an electric shock; but the fibers articulated with motoneurons or interneurons as a rule belong to many different tracts and cannot be made to conduct impulses simultaneously in response to one electric shock or any other single stimulus. Moreover, the internuncial pools are reciprocally connected by fiber paths, with the twofold result: (i) that each interneuron constitutes a link in at least one chain of several neurons, and (ii) that each chain of interneurons is linked with many others.

Synaptic transmission does not necessarily follow synaptic stimulation

The significance of these anatomical facts cannot be properly understood without considering a physiological factor. The transmission of impulses through any neuron is not an event that necessarily occurs after the activation of any synapse on the neuron. On the contrary, it is an event that fails to take place unless several synapses are activated, and, moreover, unless the activation complies with a set of most rigid and exacting conditions. Figuratively speaking, it may be said that synaptic transmission is "optional" for

† Following the example of Prof. Gerard the simple term "interneuron" will henceforth be used instead of the rather cumbersome designation "internuncial neuron."

any neuron; therefore, without detailed knowledge of the anatomical and physiological conditions that determine the "choice," it cannot be predicted whether stimulation of fibers of a certain nerve, or of a certain pathway, will
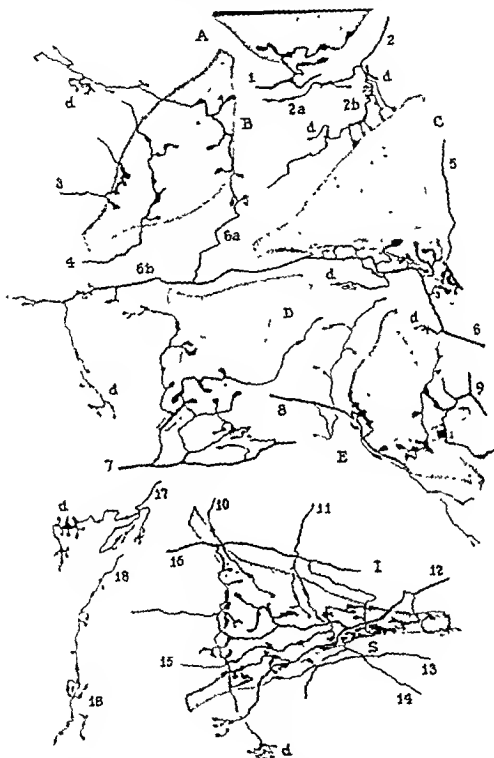
Fig. 1. Synapses on motoneurons (A to E) and on a large interneuron (I) of the spinal cord of a 15–16 day cat; *1* to *18* presynaptic fibrils. *d*, synaptic knobs in contact with dendrites. Silver-chromate method of Golgi (From Lorente de Nó, 1938c, Fig. 3).

result in transmission; nor can it be predicted through which anatomical channels the transmission, if at all, will be effected.

Study of Fig. 1 and 2 is necessary for a rigorous statement of the problem. Figure 1 illustrates the constitution of the synaptic scale on motoneurons and interneurons. *A, B, C, D,* and *E* are bodies of motoneurons; *I* is the body of

a similar large neuron which presumably belonged to the internuncial system. Numerals *1* to *16* indicate fibers forming synapses on those bodies and also synapses, *d*, on dendrites; fibers *17* and *18* form synaptic knobs only on dendrites. It will be noted: (i) that none of the fibers *1* to *16* has on any neuron more than a small number of knobs, which cover only a small part of the surface of the soma, and (ii) that the knobs formed by any one fiber are not close together, but separated by large spaces which are filled by knobs belonging to other fibers. For these reasons convergence on the same neuron of a number of presynaptic fibers results in the formation of a mosaic of knobs, which, except for narrow interstices between knobs, forms a continuous scale around the underlying soma. The interesting fact now is that although total activation of the synaptic scale of any neuron would demand that an enormous number of fibers conduct impulses, total activation of discrete zones of the scale may be effected by a rather small group of fibers. For example, in the case of cell *I* in Fig. 1, the stain of the synaptic scale is practically complete at the zone labelled *s*, so that total activation of this zone did result whenever fibers *12, 14, 15* and *16* conducted impulses. This anatomical fact, added to several experimental facts (cf. Lorente de Nó 1935*f*, 1938*c*), forms the basis for a theoretical argument.

Relevant experimental facts are: (i) Threshold stimulation of a neuron does not demand activation of all the knobs of its synaptic scale, because the stimulation by single volleys can have gradations of intensity (Fig. 5), and also because motoneurons may be caused to discharge by volleys of different constitution, *i.e.*, by impulses carried by different groups of fibers. (ii) While threshold stimulation demands convergence on the neuron of several impulses, the number of impulses is not the only determining factor. Large volleys, indeed very large ones, may remain ineffective, while a few of the impulses, which they contain, when added to impulses carried by other fibers, do set up a response. (iii) The soma of the motoneuron is electrically excitable and the nerve impulse when entering it is accompanied by an electrical sign (cf. below Fig. 14, *3*). Therefore, decremental propagation of the effects of subliminal stimulation must be expected to occur in the soma of the motoneuron. The rate of decrement is unknown but since fact ii has been demonstrated, the decrement must be high. In view of fact ii the conclusion should be that, in the case of ordinary multipolar neurons, effective summation does not take place with impulses delivered to knobs located at distances from each other greater than the distances between the knobs from any fiber, nor with a number of knobs equal to the maximal of the largest cluster formed by any one fiber (cf. the knobs of fiber *6* on cells *B* and *C* in Fig. 1). (iv) The synaptic delay cannot be reduced below a minimal value of about 0.5 msec.; this minimal interval is observed even when the effective impulses are delivered to motoneurons for which the transmission has been facilitated by the arrival of other impulses. Undoubtedly then, an impulse that has arrived at a certain synapse cannot bring to completion the excitatory process initiated at other synapses. It must start a process of its own.

In other words, impulses arriving at distant knobs may lower the threshold at a certain point, but the new impulse is not initiated unless the knob over that particular point creates its own excitatory process. (v) The effect on the neuron of impulses delivered at synapses not only must have a great spatial but also a great temporal decrement, because the effectiveness of summation of impulses arriving at different synapses, as well as the effectiveness of summation of a volley of synaptic impulses and an induction shock (Lorente de Nó, 1935*f*), declines rapidly, as the stimuli to be summated (the two volleys of impulses or the volley of impulses and the electric shock) are delivered at progressively increasing intervals of time.

Hypothesis concerning the conditions required by synaptic transmission. It seems that there is only one assumption that would satisfactorily account for these facts, namely, that threshold stimulation of the neuron takes place whenever all, or at least the majority, of knobs at a discrete zone of the neuron, as for example zone *s* in Fig. 1 *I*, are activated simultaneously, or within a very short interval of time. In agreement with this assumption it may be held, as the writer does, that the impulse is locally initiated, *i.e.* underneath the most densely activated zone of the synaptic scale, and that from there on it spreads over the remainder of the soma and also enters the axon; but other views, concerning the initiation and the spread of the new impulse would also be compatible with the basic assumption of threshold stimulation by total activation of a discrete zone of the synaptic scale. The extension of the minimal effective zone, or perhaps the density of the knobs at a zone of given extension, would depend on the instantaneous threshold of the neuron. Fact ii, however, indicates that no great reduction of the required strength of a local stimulus may be expected from the arrival of impulses to distant knobs.

Subliminal fringe. In the case of a sympathetic ganglion set in activity by electric shocks delivered to its preganglionic trunk, all the fibers of the trunk act as a homogeneous group. Therefore, the only selection that can take place in the ganglion is the number of ganglion cells responding. After a given volley, some ganglion cells will fire while others will remain in the subliminal fringe (Denny-Brown and Sherrington, 1928), the situation being that discussed by Sherrington (1931) on the basis of an illuminating diagram intended to explain the gradation of intensity of motor reflexes. But in the case of the central nervous system, where a state of rest does not seem to be possible, at least not under ordinary experimental conditions, the situation is different; in every instance the fibers having synapses on the neurons constitute several functional groups, and consequently activation of different fibers may result in a change, not only of the number of responding neurons, but also of the channels through which the transmission is effected.

Reflex reversal. This is graphically shown in the diagram of Fig. 2. Fiber *h*—which represents a number of similar fibers—has many synapses on neuron *N*, and a few on neurons a_1 and a_2. Fibers *v.a.* and *v.p.* also represent many similar fibers, each having only a few synapses on cells a_1 and a_2.

In constructing this diagram it has been assumed: (i) that activation of fiber h always results in a discharge of cell N, but does not set up a response of cells a_1 and a_2; (ii) that activation of fibers $v.a.$ and $v.p.$ fails to set up a response of cells a_1 and a_2; and (iii) that discharge of one of these cells demands stimulation by volleys containing h and $v.a.$ or h and $v.p.$ impulses. Under such conditions as these it is obvious that if fiber N is activated while fiber $v.a.$ is conducting impulses, transmission will take place through neurons N and a_1; but if fiber N is activated while fiber $v.p.$ is conducting, the transmission will be established through N and a_2. In each case the motor response will be partly different or even entirely different (reflex reversal). Transmission through N may be said to be a necessary event, it is determined by invariable anatomical moments. But transmission through a_1, or a_2 is conditioned by functional moments of great variability. In short, it is an "optional" transmission.
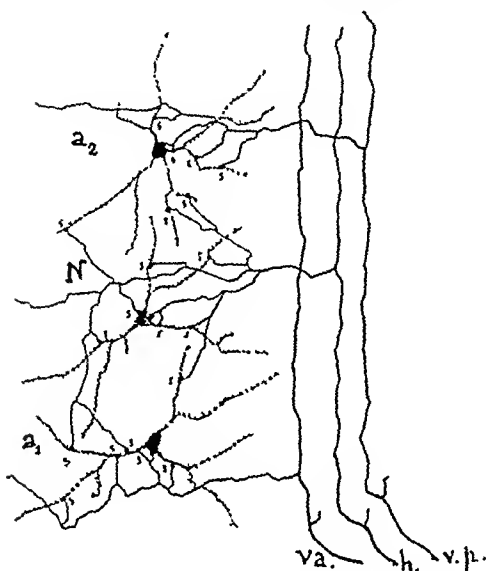
FIG. 2. Diagram explaining the reflex reversal in vestibular reflexes. Further details in text (From Lorente de Nó, 1933a).

Experimental conditions for study of synaptic transmission to motoneurons

The motoneurons of the spinal cord, or at least a number of them, can be reached directly by impulses started in a sensory nerve because, as originally described by Cajal (1894), there are collaterals of the posterior columns that reach the motor pools of the anterior horn,* but the motoneurons of the oculomotor or hypoglossal nerves have no synapses with sensory fibers. They can be reached only by impulses started in pools of interneurons. In other words, to reach these motor nuclei, impulses conducted from the periphery by the vestibular, trigeminal, glossopharyngeal, vagus, or any other nerve must cross at least one pool of interneurons, the so-called primary sensory nuclei, which, be it emphatically stated, share with the posterior horn of the spinal cord the property of having a delicacy and complexity of structure not surpassed by any other part of the nervous system, not even by the cerebral cortex. This fact must place the internuncial system of the medulla oblongata at the center of our attention. Also in the case of the spinal cord, consideration of the anatomical conditions at once reveals the extraordinary importance that must be attributed to the internuncial system. Fibers of the dorsal roots without doubt have synapses on motoneurons; but the immense majority of the articulations that they form are synapses with interneurons. If the afferent volley is such that in response to it any motoneuron fires, many interneurons must also have been caused to discharge; furthermore, as fibers of the dorsal roots form much more abundant and denser clusters of synaptic knobs on interneurons than on motoneurons, a response of interneurons must

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* Remarkably enough experimental proof, that without facilitation created' by a previous afferent volley, these collaterals actually may set the motoneurons into activity, has not been available until recently (cf. Eccles and Pritchard, 1937; Eccles, 1939).

be expected at least after any volley that activates any motoneuron, and presumably even after volleys that are too small to activate any motoneuron.† The internuncial impulses, owing to the short duration of the synaptic delays, are delivered to the motoneurons while the afferent nerve is completing its absolutely refractory period. Consequently there can be no doubt that the effects of a second volley started in the same afferent nerve or of a volley started in another afferent 0.5–0.8 msec. after the first stimulus, must be dependent on the processes that internuncial impulses have created. Under conditions such as these it may be said that a study of the responses of motoneurons after delivery of two shocks
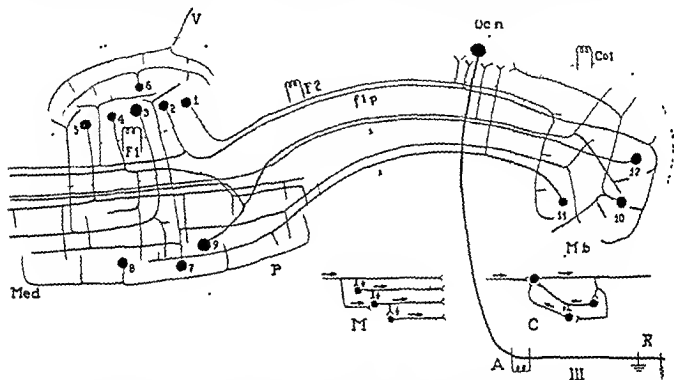
Fig. 3. Diagram of the pathways connecting interneurons among themselves and with the ocular motoneurons. V. vestibular nerve; *1* to *6*, cells in the primary vestibular nuclei; *7, 8, 9*, cells in the reticular formation in the medulla (Med.) and pons (P.); *10, 11, 12*, cells in the reticular nuclei in the midbrain (*M.b.*); *Oc.n.*, oculomotor nuclei; F1, F2 and Col., positions of the stimulating electrodes. It will be noted that by placing the electrodes on pathways of second order instead of placing them on a peripheral nerve, the passage of impulses through primary nuclei are avoided and the delivery to the motoneurons of large volleys of impulses is insured. The response of the motoneurons can be recorded with electrodes (R) from the trochlear or oculomotor nerve (III). Delivery of a shock to these nerves outside the brain stem through electrodes *A.* causes the arrival of antidromic impulses at the motoneurons.

The diagrams below illustrate the two types of chains, *M*, multiple and *C*, closed, that are found in the internuncial system. In this diagram only pathways of the vestibulo-ocular system have been included; other systems that establish synaptic connections with the ocular motoneurons are arranged according to the same plan. (After Lorente de Nó, 1938 *d.* Fig. 2, with slight additions.)

at variable intervals, or of a train of shocks to afferent nerves or central tracts, is primarily a study of the properties of the internuncial system. Information about synaptic transmis-

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† For proofs of internuncial activity in the spinal cord after arrival of dorsal root volleys, cf. the classical paper of Gasser and Graham (1933), the papers of Hughes and Gasser (1934), Hughes, McCouch and Stewart (1936) and the three recent papers of McCouch, Stewart and Hughes (1939). Although not in complete agreement with those investigators, internuncial activity is also postulated by Barron and Matthews (1938). The disagreement is, at least in part, attributable to the fact, that the recording technique used by Barron and Matthews is likely to yield records emphasizing certain potentials at the cost of other potentials (cf. the end of this report).

sion to motoneurons can be obtained solely when the complicating conditions created by the internuncial activity have been carefully considered and eliminated or at least minimized by properly chosen experimental technique.

The oculomotor preparation. An excellent opportunity for the study of synaptic transmission to motoneurons is offered by the reflex arcs that activate the ocular motoneurons, because they include a large fasciculus, the posterior longitudinal bundle and adjacent tracts (Fig. 3, *f.l.p.*), which are known to establish numerous synapses with the motoneurons. Thus, a single shock to these tracts may create a powerful volley of impulses which, after an extremely short conduction time, is delivered to the motoneurons. Extensive studies have been carried out with this system.

II. Experimental Data

Duration of synaptic delay

An important datum has been the determination of the duration of the synaptic delay (Lorente de Nô, 1935a). Figure 4 illustrates the time relations of synaptic transmission in the oculomotor preparation. In this experiment single shocks were delivered through electrodes introduced through the anterior colliculus to the level indicated in Fig. 3, *Col.* The responses were recorded from the trochlear nerve shortly after its entrance into the orbit of the eye. Weak shocks (*2, 3*) produced small discharges of motor impulses which appeared at the recording electrodes after a rather long latency; the discharges increased in size, although their latency did not markedly decrease when the shocks were strengthened up to three times threshold strength (*4, 5*). But as soon as the shock was made four times the threshold (*6*), the motor discharge showed two distinct waves (*m* and *s*), the earlier one (*m*) with a latency of some 0.7 msec. shorter than that of the second wave (*s*). Further increase of the strength of the shock (*7, 8*) up to eight times the threshold failed to alter the difference between the latencies of the two responses, but caused an increase of *m* at the expense of *s*. Finally in records not reproduced in Fig. 4 of responses to stronger shocks, the *s* wave failed to appear, obviously because all the fibers of the trochlear nerve were included in the *m* response.

The interpretation of records such as these is at present an easy matter. The shock through electrodes *Col.* (Fig. 3), when weak, stimulated only internuncial axons or cells, and the internuncial impulses thus created, after being delivered to motoneurons, stimulated some of these to discharge new impulses into their axons. The synaptically initiated motor impulses gave rise to the *s* wave. When the shock was strengthened it became capable of stimulating above-threshold elements located at a relatively great distance from the electrodes, *i.e.* motoneurons and motor axons, and these electrically initiated motor impulses gave rise to the recorded *m* wave.

Any of the records with *m* and *s* waves may be used to estimate the duration of the synaptic delay at the motoneurons. The latency, *i.e.* the shock-spike time, of the *m* wave in the recorded responses includes: (i) latency at the cathode of the motor impulses, and (ii) conduction time from the nucleus

to the recording electrode. The latency of the s wave includes, (ii) latency at the cathode of the internuncial impulses, (ii) a negligible conduction time to the motor nucleus, (iii) the synaptic delay at the motoneurons, and (iv) conduction time to the recording electrodes As the latency at the cathode must have been very nearly, if not exactly, the same for motor and internuncial impulses, it is evident that the difference between the shock-spike intervals of both waves measures with sufficient accuracy the synaptic delay at the motoneurons For example, record 9 shows that the s wave is not as synchronous as the m discharge, obviously because different motoneurons responded after slightly different synaptic delays, but still the delay was quite constant and for the majority of the responding motoneurons measured about 0.7–0.8 msec. Another important fact illustrated in Fig. 4 is that the synaptic delay cannot be reduced below a certain duration There are two distinct waves in records 6 to 9, each with its fixed latency, but there are no discharges at intermediate latencies, i e., the change in latency for those impulses that passed from the s to the m wave was not gradual, but step-like.

FIG 4 Oculomotor preparation, responses recorded from the trochlear nerve (Expt 2-II-36) Stimulating electrodes in position Col (Fig 3) The numbers on the right side of the records indicate stimulus strength in potentiometric units sh, shock artifact, m, spike attributable to direct electric stimulation of motoneurons or motor axons, s, spike attributable to synaptic stimulation of motoneurons by impulses initiated by the shock in internuncial axons or somas The undulations in the records are due to asynchronous impulses of the tonic labyrinthine innervation of the eye muscles.

Limits of variation of synaptic delay. Experiments carried out with a more delicate technique (Lorente de Nó, 1935d, e, 1938b) corroborated this conclusion, and in addition showed that the synaptic delay of motoneurons varies between very narrow limits, from 0.5–0 6 to 0 8–0.9 msec. The low figure is obtained in the case of responses to strong stimuli or responses elicited during facilitation; the high figure when the stimuli are weak or the motoneurons are in a state of partial refractoriness. Therefore, there is in synaptic transmission something similar to the all-or-

nothing law in nerve. After the arrival of impulses at its synapses, the underlying neuron either discharges within that rigidly fixed interval of time or does not discharge unless it is restimulated. The available evidence has been obtained with motoneurons and sympathetic neurons (Eccles, 1936), but there are strong reasons to believe that there are interneurons which, in this respect, behave like motoneurons.

Absolutely refractory period of synaptic arc

In other experiments on the oculomotor preparation the electrodes were placed at some distance from the motor nucleus, for example, at the position F_1 or F_2 (Fig. 3), so that it was possible to use strong shocks without danger of stimulating electrically the axons of the IIIrd or the IVth cranial nerves. With sufficiently strong shocks it is feasible, under favorable conditions, to produce volleys so powerful that all the motoneurons fire in a practically synchronous volley; then it becomes possible to measure the absolutely refractory period of motoneurons, because any response to a second volley initiated by another F shock must involve motoneurons that have recovered from absolute refractoriness. It was found (L. de Nó, 1935b) that response to a second F shock may be produced when the second shock is delivered 0.56 msec. after the first. The conclusion to be drawn from this observation is that in the whole synaptic arc there are no elements (synaptic endings or motoneurons) with an absolutely refractory period longer than that of the presynaptic axons themselves.

The antidromic shock technique. The measurement of the absolutely refractory period of the motoneuron may also be made with the antidromic shock technique (Denny-Brown, 1929) which was so successfully used in studies on the spinal flexor reflex by Eccles (1931) and Eccles and Sherrington (1931b, c, e). In addition, this technique makes it possible to determine, (a) the maximal interval during which the synaptic excitatory process remains at full value, and (b) the temporal course of the recovery of excitability during the relatively refractory period.

The antidromic shock technique is based upon the fact that nerve fibers conduct impulses in both directions. Thus, when a shock is delivered to any point of the motor nerve, it starts an impulse which travels in two directions, centrifugally toward the recording electrodes and centripetally, i.e., antidromically, toward the soma of the motoneuron. In the past there has been some discussion on theoretical grounds concerning the correctness of the assumption of Sherrington (1906) and Eccles and Sherrington (1931c) that the antidromic impulse passes through the axon hillock and penetrates into the soma of the neuron. At present, however, there can be no discussion about this point because there is sufficient direct evidence to show that the antidromic impulse enters the soma and there creates changes that have an electrical sign (cf. below, Fig. 14). It may then be taken for granted that upon delivery of a maximal shock to the motor nerve, after the proper conduction time, the soma of all the motoneurons is traversed by an impulse. The experimental evidence presently to be shown demonstrates that after the antidromic impulse the motoneuron passes through a short period, about 0.5 msec., of total unresponsiveness and then through a long period of lowered excitability.

Upper time limit during which synaptic excitatory agent remains at full value. By properly timing the delivery of the antidromic and presynaptic (F) shock it is possible to estimate the duration of the interval of time during which the excitatory processes, created by impulses arriving at synaptic knobs, remain at full value. The argument of the experiment is the following.

In a motoneuron that finds itself in a state of absolute refractoriness impulses arriving at synapses should not be expected to create any significant change until after recovery has begun. Thus, if the excitatory agent at the synaptic knob remains at full value for some time after arrival of the impulses, then a discharge will eventually occur; but if the excitatory agent has
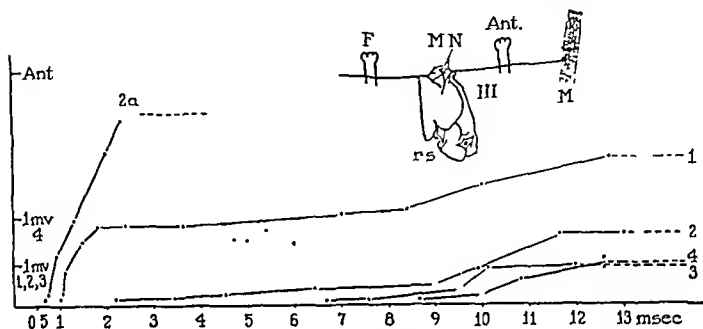
FIG 5 Oculomotor preparation, responses recorded from the internal rectus muscle (Expt 9 I-35) The diagram on top explains the conditions of the experiment F and Ant. stimulating electrodes in the positions, F_1 and A (Fig 3), $M N$, motoneurons, III, oculomotor nerve, M, muscle, $r s$, interneurons of the reticular substance In the case of curves $1, 2, 3$ and 4, one shock through the F electrodes caused responses with the height indicated by the broken lines at the right hand side of the curves The relative strength of the F shocks respectively was, in potentiometric units, 0 3, 0 17, 0 06 and 0 05 (note that the amplification was higher in the case of curve 4 than in the case of curves $1, 2$ and 3) In obtaining curve $2a$, two F shocks were used at a 0 6 msec interval, the first was subliminal for the motoneurons and the second equal to the shock used for curve 2 When delivered in succession they caused a response with the height indicated by the broken line at the right of curve $2a$ In order to obtain the curves $1, 2, 3, 4$ and $2a$, a maximal antidromic shock was delivered that caused a response of the muscle of the height indicated on the ordinate axis (ant) and afterwards at the intervals given in msec in abscissae the effective F shock The ordinates measure the height of the conditioned synaptic response Note that while in the case of curve $2a$ recovery of height was completed in less than 3 msec, in the case of curve 4 it approached completion at about 12–13 msec, and that while in the case of curve 1 some refractory neurons were capable of responding to the synaptic stimulus 1 msec after delivery of the antidromic shock, no motoneuron responded to the weaker stimulus (smaller volley) used for curve 4 until after 8 5 msec after the antidromic shock (From Lorente de Nó, 1935c, Fig 2)

a rapid temporal decrement, at the time that the motoneuron recovers from absolute refractoriness, it will be unable to cause the amount of excitation necessary to initiate a new impulse.

In the original report (L. de Nó, 1935c) it was mentioned that an antidromic shock delivered 0.43 msec. before the presynaptic (F) shock prevented the motoneurons from responding, and that therefore 0.43 msec. was the upper limit of the interval of time during which the synaptic excitatory

process remains at full value. This figure should be corrected, taking into account conduction times in the motor nerve and presynaptic fibers; but it is scarcely worth-while doing so, because the estimated duration at full value of the synaptic excitatory agent is already so small that further reduction would not increase in a significant manner its theoretical significance.

Gradation of intensity of synaptic stimuli. The lowered excitability of the motoneurons after reception of antidromic impulses reveals itself in that the size of the motor discharge in response to a presynaptic volley of given strength becomes smaller during a certain interval of time after delivery of the antidromic shock. A fundamental observation can then be made. Within obvious limits, the response regains its previous size if the presynaptic stimulus (*F* shock) is strengthened (Fig. 5). Since increase of the shock has no other result than to increase the number of impulses delivered at synapses, this result means that while activation of a certain number of synapses is sufficient to reach the threshold of a resting motoneuron, a larger number of synapses must receive impulses in order to reach the higher threshold of a neuron in a state of relative refractoriness. In other words, the results illustrated in Fig. 5 prove the correctness of the conclusion derived by Sherrington (1929) and Eccles and Sherrington (1931d) from not so direct an evidence, that the strength of a synaptic stimulus depends upon the number of active synapses. According to the number of impulses that it contains, a synaptic volley may be subliminal, *i.e.,* unable to stimulate any neuron to discharge; liminal, *i.e.,* capable of reaching the threshold of resting neurons; or supraliminal, *i.e.,* sufficient to cause refractory neurons to fire.

Rhythm of discharge of motoneurons. As already stated the presynaptic volley may be made so powerful that it causes the motoneuron to discharge an impulse into its axon immediately after completion of the absolutely refractory period. Usually, however, the rhythm of discharge of the motoneurons is low; in fact, Adrian and Bronk (1929a, b) reported rhythms of from 5 to 100 impulses per sec. for physiological discharges, and the maximal rate that has been demonstrated with the ocular motoneurons was about 300 impulses per sec. (L. de Nó, 1935g). Theoretically, a rhythm of 2000 per sec. is not impossible, and although a continuous discharge at such a rate can scarcely be expected on account of the enormous strength of stimulation that it would demand, the possibility of even only two discharges at a short interval is a fact of considerable importance (cf. later, summation of subnormality).

Recovery cycle of motoneurons

The concept of the subliminal fringe as developed by the Oxford school is based upon an anatomical factor. The afferent fibers to any pool of neurons, before building synaptic knobs, branch out with the noteworthy peculiarity that the territories of distribution of the arborizations of the various fibers overlap partially. It is, therefore, impossible to have a few neurons receiving a liminal number of impulses without other neurons re-

ceiving a subliminal volley. Furthermore, it is impossible to have a discrete number of neurons stimulated liminally without having a number of them stimulated by a supraliminal volley. From this argument it follows that the changes of height of a submaximal synaptic response are a reliable indicator of the instantaneous threshold of the individual motoneurons during the period of depressed excitability which develops after a maximal antidromic shock. Although the law of proportionality is not known, it may be stated that the number of responding motoneurons will be in direct relation to the amount of recovery of excitability of any neuron. Immediately after the
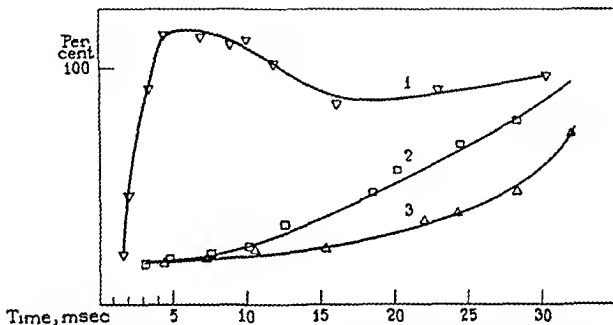
FIG 6 Oculomotor preparation, responses recorded from the internal rectus muscle (Expt. 12-VI-36) Stimulating electrodes as in Fig. 5 The curves are plots of the height of the testing response against intervals between shocks (abscissae) *1*, recovery curve of the oculomotor nerve, one maximal *Ant* response conditioning one submaximal *Ant* response *2*, recovery of a synaptic response to an *F* shock after delivery of one maximal conditioning *Ant* shock *3*, recovery curve of the same synaptic response conditioned by a series of three antidromic shocks at the frequency of 100 per sec , the conditioning interval being measured from the last antidromic shock (From Lorente de Nó and Graham, 1938, Fig 2)

absolutely refractory period only those motoneurons that receive a strongly supraliminal volley respond, while the others remain in the subliminal fringe. As recovery advances, the number of neurons leaving the subliminal fringe and reentering into the response increases; but obviously the unconditioned height will not reappear until all the motoneurons have recovered resting threshold. Thus, the antidromic shock technique allows the mapping of the temporal course of recovery of synaptic excitability in a manner similar to the recovery of electrical excitability of a multifibered nerve in terms of the height of a submaximal testing response elicited at progressively increasing intervals after delivery of a maximal conditioning shock.

The relatively refractory period of motoneurons has been investigated on several occasions (1935c, Lorente de Nó and Graham, 1938), and always with essentially identical results. The cycle of recovery (Fig. 6, *2*) includes

a single phase of depressed excitability, which under favorable conditions may be followed during 30–40 msec.; presumably it lasts for a number of additional msec. The asymptotic end of the recovery however prevents the determination of an accurate figure.

Slowed recovery after repetitive activity. It is an important fact that the course of recovery is altered by repetitive activity. Curve *2* in Fig. 6 illustrates recovery after one antidromic shock, while curve *3* represents the course of recovery after three antidromic shocks at the frequency of 100 per
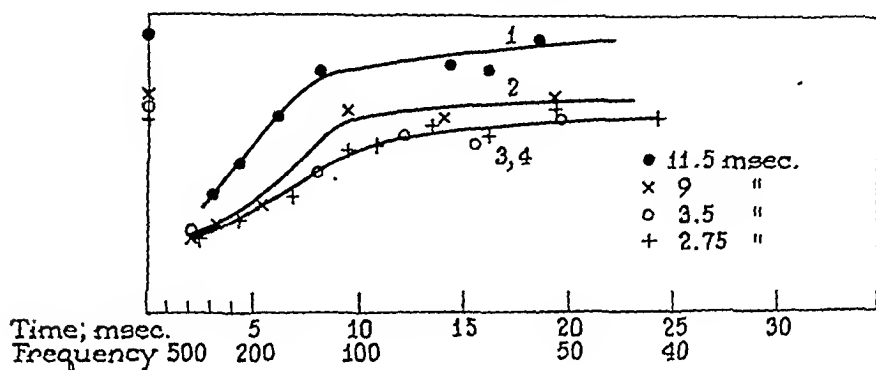
Fig. 7. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 12-vi-36). Stimulating electrodes as in Fig. 5. A synaptic response of motoneurons to two *F* shocks in quick succession, both *F* shocks being subliminal for motoneurons when delivered in isolation, is conditioned by two *Ant.* shocks at variable intervals. The interval between the second *Ant.* shock and the testing response was maintained constant (2.75 msec. for curve *4*; 3.5 msec. for *3*; 9 msec. for *2*; 11.5 msec. for *1*). Height of synaptic response plotted against the interval in msec. between the conditioning *Ant.* shocks (abscissae). Height of the synaptic response conditioned by the second antidromic shock only shown on the ordinate axis. The curves, therefore, measure the deficit of response attributable to the increase (summation) of subnormality created by two responses at the indicated frequencies, in relation to the subnormality created by one response. Note that for frequencies of response between 40 and 100 per sec. the increase in subnormality is slight, but for higher frequencies, from 100 to 500 per sec., the increase rapidly grows with the frequency. Moreover, the subnormality after two responses at the frequency of 500 per second remains practically unchanged for 11 msec. (From Lorente de Nó and Graham, 1938, Fig. 5.)

sec. It will be noted that although the early part of the recovery curve shortly after the absolutely refractory period is essentially the same in both cases, the later part of the curve is considerably lowered by repetitive activity. This finding resembles what in the case of nerve is called summation of subnormality (Gasser, 1935). It is a process of considerable theoretical significance because it may play the rôle of the "fatigue" of the neuron, so often assumed, but never experimentally demonstrated in classical neurophysiology. Subnormality, be it distinctly understood, is a special kind of fatigue. It may prevent the response to weak stimuli, but does not prevent the response to strong stimuli; and when a response is elicited, at least in blood-

perfused mammalian nerves, it has the normal size and is conducted at the normal rate (Graham and Lorente de Nó, 1938). Intense and enduring activity of nerve may be followed by a subnormality so strong that it may be called true fatigue; in this state of depression the conduction rate (Gasser, 1935) and even both the conduction rate and height of response (Gerard and Marshall, 1933) may be depressed; but according to the observations of Graham and Lorente de Nó (1938) the amount of activity necessary for that to occur is so large that it practically lies beyond the limits to be expected in physiological functioning of the central nervous system.

As in nerve (Gasser, 1935), the summation of subnormality in motoneurons has the important property of being dependent, not so much upon number, as upon frequency of impulses. In Fig. 7 it is seen that with the oculomotor neurons frequencies of less than 100 impulses per sec. produce a mild summation of subnormality, while higher frequencies cause a marked summation; the more so, the higher the frequency. In fact, as few as two responses at a frequency of 500 per sec. create such a strong subnormality that the response of the motoneurons is maintained at practically the level it has immediately after the absolutely refractory period during a considerable number of msec. Only very powerful stimuli can then produce a response. The significance of this fact has been discussed elsewhere (1938d).

Differences between recovery of motoneurons and motor axons. A striking feature of the recovery cycle of the synaptic excitability of the motoneurons (Fig. 6, 2) is that it does not parallel the cycle of recovery of electrical excitability of their axons (Fig. 6, 1). It is true that subnormality lasts in the case of both soma and axon for apparently the same length of time, but in the cycle of the axon there often is a period of supernormal excitability which has no equivalent in the cycle of the synaptic excitability of the soma. Supernormal excitability of the axon does not develop in every blood-perfused nerve (Graham and Lorente de Nó, 1938); but even then the contrast between both cycles is striking, because while the axon recovers 95 per cent or more of its electrical excitability in 4 or 5 msec. after conduction, the synaptic excitability of the motor nucleus at that time is still nearly at the same low level as immediately after the absolutely refractory period. The significance of this divergence of the recovery cycles, which also applies to stimulation of nerve by peripheral sensory endings and by electric shocks (cf. Gasser, in this Symposium), will be discussed later in relation to a model of synaptic transmission.*

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* In this connection it must be mentioned that the speed of conduction of a nerve does not become greater during the supernormal phase when the electrical excitability is enhanced, nor smaller during the subnormal phase when the electrical excitability is depressed (Graham and Lorente de Nó, 1938). In other words, changes in electrical excitability may fail to make themselves apparent in the conduction rate or in the height of the action current. Undoubtedly, the processes that determine excitability are capable to produce changes of more than one parameter of activity.

Facilitation of motoneurons and the c.e.s.

In any oculomotor preparation in which a single shock to the posterior longitudinal bundle results in a discharge of motoneurons, the size of the response increases with the strength of the shock, *i.e.*, with the number of impulses in the presynaptic volley. Occasionally the response grows up until all the motoneurons are engaged, but usually the maximal response to a single shock of any strength includes only a fraction of the total number
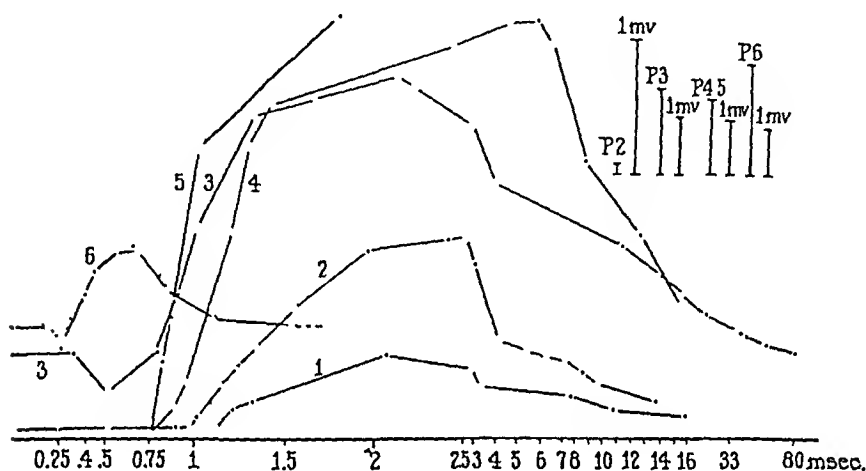
Fig. 8. Oculomotor preparation; responses recorded from the internal rectus muscle. The ordinates measure the height of the response to an *F* shock when conditioned by another *F* shock at the interval indicated in msec. in the abscissae.

1–5. (Expt. 18-XI-34). Vestibular nuclei destroyed and also a transverse section in pons as in Fig. 15, *I* in Lorente de Nó, 1933*b*, Fig. 16 in that paper shows that as a consequences of the lesion the vestibular after discharge was enormously prolonged; a similar process may explain the unusually long duration of facilitation in curves *1* to *5*. The testing *F* shocks alone produced responses with height P_2, P_3, $P_{4,5}$ (cf. upper right corner). The ratio of strengths of the conditioning and testing shocks were: *1*, 40/100; *2*, 50/100; *3*, 40/100; *4*, 160/100; *5*, 120/100. In *4* the testing shock was just maximal, in *5* supramaximal (125/100).

6. (Expt. 19-XI-34). Intact medulla. Conditioning shock just above threshold. Testing shock 250/100 larger. Note facilitation starts at a 0.4–0.43 msec. interval between shocks, and lasts for about 1 msec. while in *3* it lasted for 80 msec. 1 mv. = one millivolt. (From Lorente de Nó, 1935*d*, Fig. 1.)

of motoneurons. In such cases it is possible to obtain a further increase, indeed it is often possible to cause all the motoneurons to fire almost synchronously, by delivering to the posterior longitudinal bundle, instead of one, two successive shocks of which the first or conditioning shock may be subliminal, *i.e.* so small that the volley of impulses produced by it does not reach the threshold of any motoneuron. It is obvious that the conditioning volley, whether liminal or subliminal, creates some process which "facilitates" the stimulation of motoneurons by the second or testing volley. The temporal course of facilitation is best represented by "facilitation curves"

obtained by plotting the size of the testing response which is proportional to the number of responding neurons against the interval between shocks. A number of facilitation curves obtained with the oculomotor preparation have been reproduced in Fig. 8 and 9 (cf. also Fig. 13).

The concept of the central excitatory state (c.e.s.) Facilitation in the oculomotor preparation is entirely comparable to the well-known phenomenon
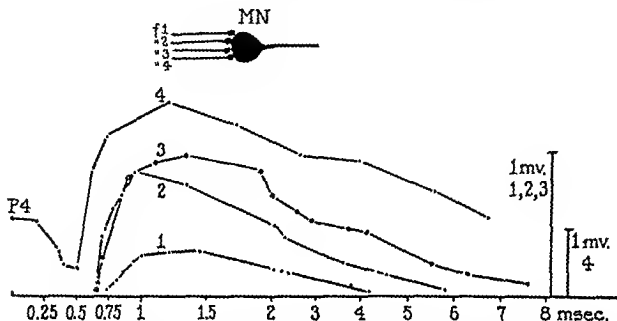
Fig. 9. Oculomotor preparation; responses recorded from the internal rectus muscle. Height of the response to the testing *F* shock (ordinates) plotted against the interval in msec. by which it follows the conditioning shock. *F* is the testing response.

1, 2, 3 . . . . destroyed . . . . of conditioning and testing shocks: *1*, 50/100; *2*, 60/100; *3*, 160/100.

4. (Expt. 7-I-35). Intact medulla. Subliminal conditioning shock 60/100 of the testing shock. *P₄*, height of the unconditioned testing response. The diagram on top explains the depression of the testing response in curve *4* at intervals between shocks of more than 0 25 and less than 0.5 msec. The conditioning shock is supposed to have stimulated fiber *f1* and the testing shock, when delivered in isolation, fibers *1* to *4*. When both shocks were delivered in succession at intervals of less than 0.5 msec., the testing shock stimulated fibers *f₂, f₃* and *f₄* only, because fiber *f₁* had responded to the conditioning shock. Therefore, as soon as the detonator action of impulse *f₁* began to decay, the response to the testing shock was depressed. (From Lorente de Nó, 1935*d*, Fig. 2.)

of the facilitation of reflex responses in the spinal cord and should be explained in similar terms. A suggestive explanation of facilitation was offered by Sherrington (1925; cf. Fulton, 1926; Bremer, 1930; Eccles and Sherrington, 1931*d*). Facilitation was believed to depend upon the creation in the motoneurons of a certain process called c.e.s. (central excitatory state) characterized: (i) by its being enduring, *i.e.*, its being dissipated at a relatively slow rate, and (ii) by its being capable of summation. In certain respects c.e.s. was compared with Lucas' (1917) local excitatory process in nerve. Each subliminal volley of impulses would create a certain amount of c.e.s. and summation of successively produced quanta would eventually yield the amount necessary to reach the threshold of the motoneuron and

set up a discharge. According to this assumption, which has been one of the most fruitful working hypotheses ever introduced in neurophysiology, facilitation would take place within the motoneurons, or in general, within the individual neurons.

Insufficiency of concept of c.e.s. for complete explanation of known facts. The creation in subliminally excited neurons of a process of the nature assumed for the *c.e.s.* has never been disproved, but recently it has become apparent that the hypothesis of *c.e.s.*, as originally presented, is not sufficient to explain newly acquired facts. In the first place, if effective excitation were
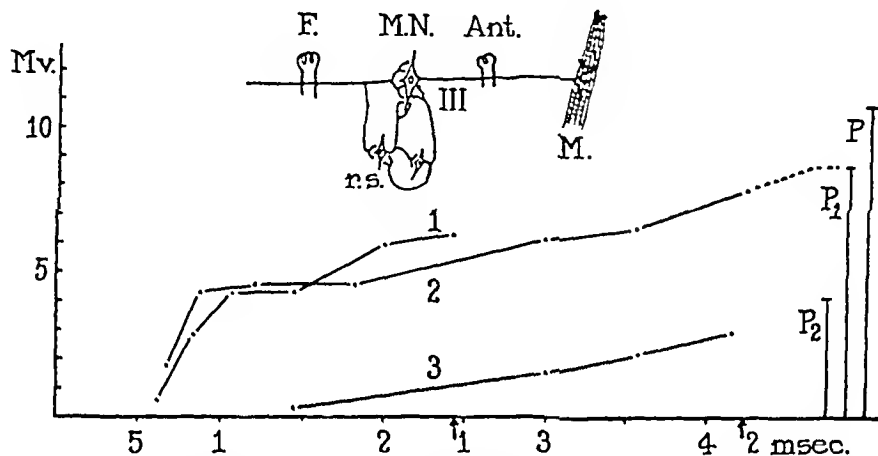
FIG. 10. Oculomotor preparation; responses recorded from the internal rectus muscle (Expt. 7-I-35). Stimulating and recording electrodes as in Fig. 5. The curves are plots of the response to a testing F shock with unconditioned height P_2, when this response was conditioned by a maximal antidromic shock (3) or by a maximal antidromic shock and a conditioning F shock (1, 2). The response to the testing F shock, when conditioned only by the conditioning F shock, was facilitated and its height became P_1. The fixed interval between both F shocks is indicated by arrows 1 and 2 below the abscissa axis. The abscissae measure the interval between the antidromic and the second F shock. Therefore, the antidromic shock was delivered first between both F shocks, then simultaneously with the conditioning F shock and finally before this shock. Comparison of curve 3 with curves 1 and 2 show that the antidromic shock at no moment prevented the creation of or destroyed all the facilitation that had already been created. P_i, height of the response to the antidromic shock (From Lorente de Nó, 1935c, Fig. 4).

due to summation of successively produced quanta of *c.e.s.*, the synaptic delay could not have the rigidly fixed limits that it has, especially not its relatively long minimal duration. On the contrary (cf. Eccles and Sherrington, 1931*a*), the synaptic delay should vary between wide limits and should be reduced to almost nothing in facilitated responses. Then, according to the *c.e.s.* hypothesis, the subliminal changes underlying the state of facilitation should be destroyed by the all-or-nothing response initiated by the entrance into the motoneuron of an antidromic impulse. In fact, however, facilitation is not destroyed by the entrance of an antidromic impulse into the motoneuron (Lorente de Nó, 1935c).

Facilitation created during absolute refractoriness and facilitation persisting after an all-or-nothing response. While delivering a maximal antidromic shock to the motor nerve at various intervals before the conditioning shock or between the conditioning and the testing shock (Fig. 10) it was found (1935c) that facilitation may be created when the conditioning volley finds the motoneurons in a state of refractoriness, even of absolute refractoriness; and also facilitation was not destroyed by the entrance of an antidromic shock into the soma of the motoneurons, although, as already indicated, the antidromic impulse by initiating an all-or-nothing response of the soma must have wiped off any enduring change initiated by the subliminal conditioning volley. The conclusion drawn from this experiment was that the antidromic volley had no effect on facilitation except by its depression of the excitability of the motoneurons. This conclusion might have been somewhat too radical because the experiment does not prove in a conclusive manner that enduring subliminal changes were not produced in the motoneurons by the conditioning volley; but the conclusion was strongly supported by the absence in curve *2* in Fig. 10 of an apparent discontinuity at the point of simultaneous delivery of the antidromic and the conditioning shocks. Indeed, in view of curve *2* in Fig. 10 it must be stated that if an enduring subliminal change had been created in the motoneuron by the conditioning volley, its participation in maintaining facilitation was small in relation to the effect of some other agent for facilitation which could not be reached by the antidromic impulse and therefore was located outside the motoneuron.

Extracellular agents for facilitation. Several extracellular agents may be considered. First, it may be assumed that a chemical agent is released by the synaptic endings, and remaining outside the motoneuron, is not affected by the antidromic impulse. In former times, and in relation rather to long lasting responses (after discharge) than to facilitation, the possibility of enduring chemical changes at the synapses was considered by Fulton (1926), who later (1938), after taking into account newly acquired evidence, has expressed the view that, as the arguments underlying the original hypothesis have lost a great deal of their force, long-lasting excitation can be explained chiefly in other terms. Rosenblueth (1934) and Forbes (1934) were strongly in favor of chemical agents; but, it seems that these views too have become largely superseded (cf. the views held by Cannon and Rosenblueth, 1937). The problem of extracellular agents for facilitation has, however, recently acquired a new aspect, because Barron and Matthews (1938) have developed a theory which, although considering chiefly unspecific ions, still leads to the assumption of extracellular agents for facilitation that, with respect to the temporal course of their action, could be compared with chemical agents. Barron and Matthews believe that long-lasting differences of potential between the synaptic endings and the parent fibers set up changes of ionic concentration in the neighborhood of the soma of the nerve cells, thus creating in them a certain degree of depolarization which eventually may lead to a rhythmic discharge of impulses.

Extracellular agents of this type would create facilitation and would not be accessible to antidromic impulses. Thus, its existence cannot be excluded on the basis of the experiment of Fig. 10. Moreover, environmental changes, chemical or only of concentration of unspecific ions, must now be considered as possible agents for facilitation because there is factual evidence brought forward by Dusser de Barenne, McCulloch and Nims (1937; see also Dusser de Barenne and McCulloch, 1939) that during activity changes take place in the nervous system which make themselves evident by changes of pH and lead to alterations of excitability that parallel those known to occur in nerve when the pH of the medium is varied (Lehmann, 1937). In this connection the experiments of Gerard and collaborators (cf. Gerard, 1936) must also be cited, as reference must also be made to important work on the effect of chemical agents in the response of sympathetic ganglia (cf. Bronk, in this Symposium).

There can be no doubt that changes in the environment of the nerve cells, no matter what might be their origin, will alter the excitability of the neurons. Thus, these changes may eventually result in a lowering of threshold and facilitation of the responses to a given stimulus. The question is, therefore, whether environmental changes are the only mechanism underlying facilitation and, if not, whether they are the primary mechanism.

Impossibility of explaining facilitation solely in terms of chemical change. Proceeding along a line of argument similar to that employed by Eccles and Sherrington (1931e) in their discussion of inhibition by enduring chemical agents, the following remarks must be made (cf. Lorente de Nó, 1936). Facilitation by chemical agents released by impulses at the synaptic endings should follow a temporal course of quite definite configuration; in fact, the chief argument adduced by Rosenblueth (1934) in favor of chemical agents was derived precisely from a comparison of the temporal course of central responses with the course of peripheral responses believed to be due to chemical reactions. Thus, if facilitation were due *only* to changes created by the conditioning volley in the neighborhood of the motoneurons, then no essential differences in the course of facilitation should be observed in different preparations, nor in the same preparation when conditions are altered at points distant from the facilitated motoneurons. There is no doubt that suitable and permissible assumptions could be made in order to interpret any of the curves in Fig. 8 and 9 in terms of the creation and dissipation of chemical agents or of changes of ionic concentration in the environment of the neurons: but there is also no doubt that the assumptions made, for example, for the case of curve *1*, Fig. 8, cannot apply to curve *6* in the same Fig. 8. Nor can any assumption capable of accounting for curve *3* in Fig. 8 explain the curves in Fig. 13. Again, assumptions made for the facilitation of motoneurons of the oculomotor nucleus would not be suitable for the motoneurons of the hypoglossus nucleus, etc. The differences in the time of onset, rate of ascent, position of maximum height, duration and rate of descent found in the facilitation curves are so enormous that they demand at least

the additional assumption of some other mechanism of facilitation. In other words, these great differences indicate that whether chemical changes took place in the neighborhood of the motoneurons immediately after arrival of the conditioning volley or not, and whether the changes persisted for some time and may account for part of the facilitation or not, still some other extracellular mechanism was operative in maintaining facilitation. The additional factor has proved to consist of subliminal stimulation of motoneurons by a long lasting stream of internuncial impulses (internuncial bombardment).

Restimulation of motoneurons by subliminal volleys of internuncial impulses. The idea that interneurons reenforce the transmission of the current of "nervous energy" effected by simple and direct pathways was originally stated by Ramón y Cajal (1901; 1911, p. 150) who, from his own anatomical discoveries, concluded that the interneurons are arranged in parallel chains superimposed upon the simple two-neuron arcs. Several illuminating diagrams may be found in Cajal's monumental work (1911, Fig. 103 and 104; reproduced in Lorente de Nó, 1933b, Fig. 2). In contemporary neurophysiology the concept of long-lasting stimulation (after discharge) by impulses delayed during their passage through internuncial synapses was introduced by Forbes (1922), and elaborations of this concept were suggested by Bremer and Rylant (1926), Ranson and Hinsey (1931), Forbes, Davis and Lambert (1931), the present author (1932) and others. Eccles and Sherrington (1931c, e), in order to account for experimental results obtained in studies on after-discharge and sustained inhibition of the flexor reflex, also postulated the existence of a bombardment of motoneurons by internuncial impulses. In this connection it might not be impertinent to mention that, no matter how much complexity of structure could be attributed to the nervous system, still the possibility of the postulated internuncial activity was not established* (cf. Eccles, 1936, p. 395) until it was demonstrated that the internuncial circuits described by Ramón y Cajal (1911, Fig. 103 and 104) were not peculiar to parts of the nervous system of highly specialized structure, *i.e.*, the cerebral and cerebellar cortices, as he had stated (1911, p. 150-151) but with few exceptions are present everywhere in the central nervous system (Lorente de Nó, 1933b). Experimental studies (*Idem.*, 1926, 1928; summary 1931) had previously brought to light a number of facts which, as it seems (cf. Sherrington, 1934), conclusively demonstrated the participation of the internuncial system in the establishment of motor reflexes. One of these facts was (1928, p. 105-107; cf. 1933b, Fig. 18) that the duration of the response to a given peripheral stimulus depends upon the activity of internuncial relays. Thus, it was not difficult to arrive at the conclusion (1935c, Fig. 2, 1935d) that facilitation of motoneurons is the result of continued bombardment of the motoneurons by internuncial impulses arranged in volleys of subliminal density, which summate with the impulses initiated by the testing volley.

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* Reference must be made to the review, that Forbes published in 1934 (p. 188-190).

Period of effective summation of impulses arriving at different synapses. Before attempting an analysis of the internuncial activity that underlies facilitation and after-discharge, it is necessary to determine the duration of the period of effective summation of nerve impulses delivered to different synapses on the motoneuron. For this purpose two methods have been used: (i) fractionation of a synchronous volley of impulses into two volleys delivered in succession at different intervals (1935*d*, Fig. 2, *4*), and (ii) delivery to the motor nucleus of two volleys carried by different fibers (1935*e*). Experiments in which the summation of subliminal volleys of impulses with induction shocks were investigated (1935*f*) led to similar conclusions.

The conclusion may be expressed in the following statement. When two volleys of impulses are delivered to different synapses on a motoneuron, the statistical chances of effective summation are greatest if the volleys are delivered simultaneously or at intervals of less than 0.15 msec. They decrease rapidly when the volleys are separated by progressively increasing intervals of time, because some impulses fail to summate when they have arrived at intervals of over 0.15–0.2 msec. Finally, the chances of effective summation disappear when the separation between volleys becomes as small as 0.5 msec.

Possible existence of a second period of lowered threshold of motoneurons after subliminal synaptic stimulation. Eccles (1936, 1937) working on the transmission of impulses through the superior cervical ganglion obtained, with the fractionation method and the double volley technique, results similar to those reported with the ocular motoneurons and therefore concluded that the nerve impulse upon its arrival at the synapse creates an excitatory process of extremely brief duration, the "detonator" response which, if sufficiently strong, after completion of the synaptic delay leads to the discharge of a new impulse by the ganglion cell. The detonator action of the nerve impulse would be identical with what the present author called "synaptic excitatory process" (1935*c*, *d*; cf. Eccles, 1939, p. 368). In addition, Eccles made the important discovery of a *second* period of lowered threshold of the ganglion cell, which would develop after dissipation of the detonator action. In order to account for the second period of lowered threshold, Eccles assumed the production in the neuron of a certain change which he termed *c.e.s.* There is, however, an essential difference between Eccles' concept and the original concept as developed by Sherrington (1925) and by Eccles and Sherrington (1931*d*). According to Eccles, *c.e.s.* should only modify the threshold of the ganglion cell without ever reaching sufficient strength to initiate the discharge of an impulse. The initiation of a new impulse would demand the arrival of new impulses to synapses and the creation of their detonator actions.

Spatial and temporal summation

Eccles' observations are of fundamental importance because they reopen the question of how far facilitation may be attributed to protracted changes in threshold in individual neurons. The question is indeed an essential one. If nerve impulses arriving at synapses should develop only detonator actions which are no longer in duration than the absolutely refractory period of presynaptic fibers, then temporal summation of impulses arriving in succession through the same fiber would be impossible, and in the nervous system no other type of summation could exist than the spatial summation of impulses arriving simultaneously or within a short interval of time through different synapses. But if subliminal impulses should produce a second phase of lowered threshold, then temporal summation of impulses conducted in succession by the same fiber would occur.

Second phase of summation in different structures. In view of the results obtained by Eccles with ganglion cells, the temptation is great to assume the development of c.e.s. in subliminally stimulated motoneurons. It is indeed great because processes comparable to those observed in the ganglion cells have been demonstrated for other structures. The following recent observations need mention here. In the partially curarized neuromuscular junction of the frog one impulse may fail to cross the junction, but it creates a certain enduring process, which enables a second impulse to cross the junction (Bremer, 1930; Bremer and Homès, 1932). An impulse may fail to cross an anodal block in nerve, but it causes an enduring change which enables a second impulse to cross the block (cf. Erlanger, in this Symposium). Stimulation of an adequately treated nerve by a train of shocks at low frequency results in a progressive increase of the number of responding fibers (recruitment), which undoubtedly proves the existence of a long period of lowered threshold in nerve fibers subliminally stimulated by electric shocks (Gasser, 1938). In agreement with this conclusion a second phase of enhanced excitability may be directly demonstrated in blood-perfused mammalian nerve by stimulation with a train of subliminal shocks of high frequency. After the last shock of the series the nerve successively passes through: (i) a short lasting period of lowered threshold, which corresponds to the period of local summation of subliminal shocks of Lucas and Adrian (1917); (ii) usually postcathodal depression, but sometimes only return to normal excitability; and (iii) a long-lasting second period of lowered threshold (Lorente de Nó, quoted by Gasser, 1938). Similar effects, except for the lack of postcathodal depression, may be observed after a single shock in anodally polarized nerves (Blair, 1938a).

With data such as these it is not surprising that the consideration of a second period of summation for the interpretation of facilitation of central neurons has been demanded by a number of investigators (Eccles, 1936, 1939; Gasser, 1938). It is even understandable that, despite the absence of conclusive evidence, the demand has been made in emphatic terms (Bremer and Kleyntjens, 1937).

Failure to demonstrate second phase of summation in motoneurons. It has been impossible, however, to demonstrate in a convincing manner the development of a second phase of lowered threshold in subliminally stimulated ocular motoneurons. This lack of success does not prove that a second phase of summation did not develop; as a matter of fact, it proves only that the lowering of threshold due to such a process is small in relation to the change in threshold produced by alteration of the intensity of the internuncial bombardment, and therefore cannot be demonstrated if a variation in the strength of the internuncial bombardment takes place. There is experimental evidence in support of this conclusion.

The records in Fig. 11 are an excellent illustration. In this experiment two different electrodes were used to create volleys of presynaptic impulses. One shock was delivered through electrodes *F* (Fig. 3) and the other through electrodes *Col.* (Fig. 3). In isolation

the weak F shock set up responses (Fig. 11, *3, 5, 18*) composed of several waves, the first one due to impulses set up in fibers of the posterior longitudinal bundle, and the following responses due to impulses delayed during their passage through internuncial neurons (cf. 1938*d*, Fig. 3). The C shock, however, caused a response composed of an almost synchronous spike, which was followed by a temporary cessation of the labyrinthine tonic
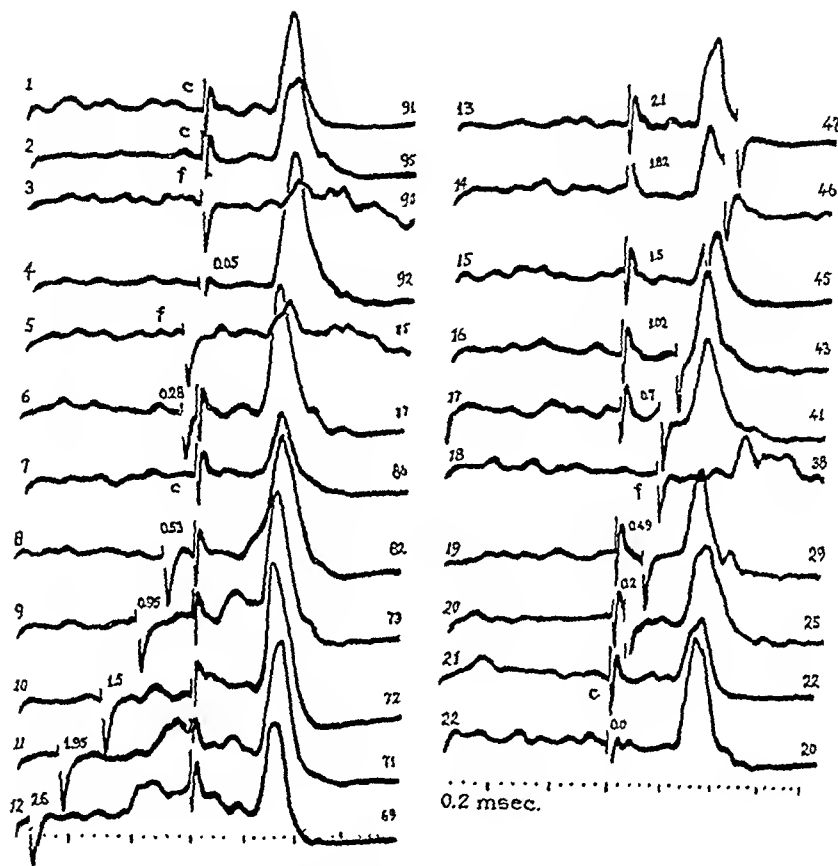
FIG. 11. Oculomotor preparation; responses from the trochlear nerve. (Expt. 2-II-36). Two stimulating electrodes in positions F_1 and Col. (Fig. 3). The responses to the C and F shocks in isolation have been reproduced in records *1, 2, 3, 5, 7, 18* and *21*. There was a certain variation of height of the C response due to the discontinuous character of the tonic labyrinthine innervation, the response being of course larger when the *c* impulses happened to coincide with a large internuncial volley. Record *1* reproduces the largest observed response to a C shock in isolation. The numbers on the right hand side of the records indicate the order in which they were obtained; between each two consecutive records there was an interval of two seconds. For records *4* to *12* the F shock preceded the C shock at the interval indicated in msec. on the records. For records *13* to *22* the C shock preceded the F shock. Time in 0.2 and 1 msec. below (from Lorente de Nó, 1938*d*, Fig. 7).

discharge usually present in electrograms of the eye muscles or their nerves. As the spike included only a fraction of the trochlear motoneurons, this silent period cannot be ascribed to refractoriness of motoneurons. The F and the C shocks may have in part stimulated the same fibers, but there can be no doubt that they also stimulated different fibers, because when delivered simultaneously (*22*) more motoneurons discharged than when either

shock was delivered in isolation When the F preceded the C shock, the response to the latter was facilitated (4 to 12), the period of facilitation lasting through the duration of the F response The facilitation curve in this case did not show a trough around the 0 5 msec interval Apparently the weak F shock set up impulses after various latencies and the c impulses always met in the nucleus some f impulses with which they could summate

The results were entirely different when the C preceded the F shock At an interval of 0 2 msec (20) the late waves of the F response were greatly reduced and even the early wave, which was superimposed upon the descending phase of the C response, was reduced in size At the 0 49 msec interval (19) the F response was reduced to a small initial wave At greater intervals, up to 4 msec, it was totally abolished, as was also the case when the C response included only a small number of motoneurons (15) The F response began to reappear at an interval of about 4 msec between shocks and up to the last interval studied, 5 msec, it did not show signs of being facilitated

Since there was response to the F shock at the 0 2 and 0 49 msec intervals when the C response was large and there was no F response at longer intervals (15) when the C response happened to be small, the absence of F response at intervals of over 0 5–0 7 msec cannot be ascribed to the failure of the F shock to set up impulses in presynaptic axons that had been made refractory, nor can it be ascribed to refractoriness of motoneurons included in the C response The only possible explanation would be the following The C shock created a volley of impulses (c) which was delivered to the motoneurons, and at the time that the motoneurons were responding a temporary cessation of the internuncial bombardment, responsible for the tonic labyrinthine innervation, took place This interruption of the internuncial bombardment resulted in the silent period in the electrogram of the trochlear nerve The c volley stimulated a number of motoneurons above threshold and other motoneurons subliminally As long as the synaptic excitatory processes created by the c impulses were able to summate with those created by the f volley, a number of motoneurons fired in response to the F shock and produced an f wave in the recorded response, but as soon as these detonator actions were dissipated, since the tonic background of excitation had been suppressed, the f impulses remained ineffective This happened about 0 5–0 7 msec after delivery of the C shock Afterwards no response to the F shock was observed until the tonic labyrinthine innervation was reestablished Therefore, the conclusion is unavoidable that if the e impulses had produced in subliminally stimulated motoneurons a second period of lowered threshold, or had resulted in the release of chemical agents, or in changes of ionic concentration in the environment of the motoneurons, all these effects were weaker than the rise in threshold due to the cessation of the indeed mild internuncial bombardment responsible for the labyrinthine tonus Hence there resulted the temporary inhibition (extinction) of the f response

Upper limit of lowering of threshold possibly attributable to c e.s. or environmental changes with brief stimulation. If it were permissible to apply to motoneurons quantitative results obtained with motor axons, then it could be stated that the lowering of threshold that might be expected during the second phase of summation (c e s. of Eccles) is no more than 4–5 per cent of the resting threshold, because this is the maximal increase in excitability ever observed during the second phase of summation created in the trochlear nerve by a rhythmic series of subliminal induction shocks (unpublished experiments). A similar upper limit can be estimated from the results of other unpublished experiments in which the excitability of the motoneurons was tested with induction shocks. Internuncial bombardment easily produced a lowering of the electrical threshold of motoneurons amounting to 50 per cent or more of the resting value.

The ability of the internuncial bombardment to mask the effect of other possible agents for facilitation is also shown by other observations. Facilitation attributable to a second phase of summation of the motoneurons, judging by what is known from ganglion cells, neuromuscular junctions and

nerve fibers, should start rather late and have a gradual onset. For example, with the trochlear nerve, the second phase of summation does not start earlier than 1 msec. after delivery of the last conditioning shock. A similar late and gradual onset should be expected for facilitation due to changes in ionic concentration in the environment of the neuron (Barron and Matthews, 1938). But facilitation due to internuncial bombardment begins early, *i.e.* as soon as synaptic delay at interneurons has been completed, and under favorable conditions it may be expected to have a sudden onset, reaching maximal value in a fraction of a millisecond. An early and sudden onset is frequently observed (Fig. 8, 9, 13), and since at intervals between shocks of 0.43–0.75 msec. facilitation can scarcely be attributed to anything but internuncial bombardment (*i.e.* to instantaneous summation of detonator actions), it is obvious that the internuncial bombardment is a powerful agent for facilitation. Consequently, if the internuncial bombardment is maintained, the effect of other facilitatory mechanisms will scarcely be detectable until the bombardment ceases (cf. Dusser de Barenne and McCulloch, 1939, p. 334).

The conclusion to be drawn from the preceding remarks is, therefore, the following. Whether in subliminally stimulated motoneurons after dissipation of the detonator actions a second phase of lowered threshold develops, and whether in the immediate neighborhood of the motoneurons chemical agents are released, or changes in ionic concentration take place, which result in a lowering of threshold of the motoneurons,—these are still unsolved questions. In view of the available evidence it seems likely that processes such as these will be demonstrated with new techniques, but it must be expected that the lowering of threshold attributable to them will amount to a few per cent of the resting threshold, and therefore will be small in relation to the great lowering that may be produced by the detonator actions of the impulses of the internuncial bombardment (cf. Fig. 8, 9 and 11). For this reason, in theoretical arguments intended to represent only a first approximation, it is permissible to explain facilitation solely in terms of the short synaptic excitatory processes (detonator actions) created by the impulses of the internuncial bombardment (cf. Lorente de Nó, 1938*d*, p. 221 and 228). The possible existence of other agents for facilitation will have to be considered from the first moment in those cases in which interneurons are not present, as for example, in the cases of sympathetic ganglia, the dentate nucleus of the cerebellum, the nuclei of Goll and Burdach, the superior olive, etc. Unfortunately, except for studies on sympathetic ganglia, the course of facilitation in relays of this type has not been investigated. On the other hand, it must be considered that enduring intra- or extracellular changes, especially cumulative changes, will not only lead to modifications of the internuncial bombardment, but will also be the only factors capable of explaining facilitation or inhibition (extinction) remaining after cessation of enduring bombardments of neurons by nerve impulses (cf. Dusser de Barenne and McCulloch, 1939; Bronk, in this Symposium).

Mechanisms underlying internuncial bombardment

If prolonged excitation is maintained in the neurons by repeated creation of ephemeral excitatory processes, then there must be in the nervous system mechanisms capable of producing bombardment of the neurons at high frequency with volleys of impulses of subliminal density, *i.e.* with volleys that activate, on any one neuron, synapses, which being scattered all over the soma, cannot produce threshold atimulation. A neuron stimulated in manner will actually have a low threshold, because a few additional impulses will be sufficient to cause total activation of discrete zones of the synaptic scale and therefore initiate a new impulse (cf. Fig. 1). The bombardment of the motoneurons is a result of the activity of the chains of interneurons (cf. 1938*d*). An open chain like *M* in Fig. 3, provided that the impulses are able to cross the successive internuncial synapses, will cause bombardment of the motor nucleus at high frequency. That such chains actually exist and are active under physiological conditions was demonstrated some time ago (L. de Nó, 1928, p. 160), but open chains can maintain internuncial bombardment for only short intervals of time.

Enduring bombardment obviously requires repetitive passage of impulses through the same interneuron; therefore, it requires the existence of closed chains such as *C* in Fig. 3. Their existence has been implicitly accepted by several authors, Forbes, Cobb and Cattell (1923), Bremer and Rylant (1926), and more specifically by Ranson and Hinsey (1930) and the present author (1932, 1933*b*, 1934). As the absolutely refractory period of the neuron is about of the same order of magnitude as the synaptic delay, a closed chain with only two neurons may theoretically maintain the circulation of impulses (1934); practically, however, summation of subnormality, or in this specific case, true fatigue, will prevent the circulation after passage of very few impulses. Of course, if the chain includes a considerable number of links, the frequency of activation of each neuron will be sufficiently low to permit a prolonged circulation of impulses. Direct proof of the circulation of impulses through closed chains is not as yet available, but it is hardly necessary, because for conclusive anatomical reasons enduring bombardment unavoidably requires circulation through closed chains; therefore, as soon as the existence of bombardment has been demonstrated, circulation must be postulated.

The circulation may be thought of as being the result of automatic activity, *i.e.* of being created within the chain itself, hence the terms "reverberation" (Forbes, Cobb and Cattell, 1923), "reverberating chains" (Ranson and Hinsey, 1930) and "closed self-reëxciting chains" (Lorente de Nó, 1933*b*). Automatic activity may be assumed for chains with a large number of links, but for shorter chains it is easier to believe (cf. *Idem.*, 1938*d*, p. 230) that circulation is not automatic, but is maintained by impulses arriving from the periphery, or from other parts of the nervous system which find themselves in a state of activity.

A possible mechanism is illustrated in Fig. 12. Let it be assumed that fiber f_2 is conducting a series of rhythmic impulses initiated, for example, in a peripheral end-organ such as the labyrinthine maculae. The f_2 impulses are able to stimulate neuron 2 above threshold, but they stimulate neurons 1 and 3 subliminally. If now fiber f_3 is made to conduct an impulse which happens to be synchronous with one of the f_2 impulses, neuron 3 will fire, with the result that the impulses fed back to neuron 3 through chain C will summate with successive f_2 impulses and cell 3 will henceforth be able to conduct every impulse arriving through fiber f_2. Circulation in chain C and transmission through cell 3 will stop when any of the links in the chain, owing to repeated activity, acquires a high threshold and fails to transmit the circulating impulses. If the rise in threshold is created by an extra impulse arriving through a fiber like d, then the process may be called active inhibition (*Idem.*, 1938d, p. 238; cf. Gasser, 1937c).

Indirect evidence of internuncial activity. By recording the responses of the motoneurons after conduction through the motor nerve, only indirect proofs of the activity of the internuncial system can be obtained. There is no need of presenting the available evidence again in this report, since it has been recently described (Lorente de Nó, 1936, 1938b, c, d). Only one point will be emphasized here.

Modification of the activity of the internuncial mechanism readily explains all the characteristics of the facilitation curves (Fig. 8, 9, 13). The variability of the facilitation curves observed in different preparations, or even in the same

FIG. 12. Diagram explaining the production of reflex reversal by concurrent stimulation of two fibers (fibers f_1 and f_2 or f_2 and f_3) from different peripheral sense organs and its maintenance by the impulses conducted by the closed chain C, after fiber f_3, which initiated the response of cell 3, ceases conducting. Maintenance of response through 3, in the absence of impulses conducted by fiber f_3, would be called central after discharge. After discharge would cease as soon as the neurons in the closed chain C should acquire subnormal threshold. But if the subnormal threshold should be created by an extra discharge of cells of chain C caused by impulses conducted by collateral d, the phenomenon would be called active inhibition. Each one of the links in the closed chain represents a multiple chain of neurons such as chain M in Fig. 3 (from Lorente de Nó, 1938d, Fig. 12).

preparation under different conditions, finds a plausible explanation in the fact (*Idem.*, 1928, 1933b) that changes in the activity of the chains of interneurons may result in most varied alterations of the response to a given stimulus. This fact is indeed not surprising when it is realized that

synaptic transmission through any chain of neurons is optional (Fig. 2) and that the internuncial system includes many thousands of neurons (cf. *Idem.*, 1938d, Fig. 1); it certainly has many hundred times more neurons than the motor nuclei.

Thus, there is no difficulty in explaining why, although facilitation usually begins 0.5–0 6 msec. after delivery of the conditioning shock, it may sometimes start earlier, at 0.4–0.5 msec., and at other times much later, at 1–2 msec. The response to the testing shock becomes larger when the testing
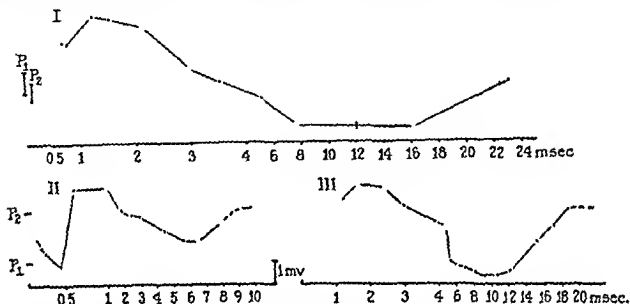
FIG 13 Oculomotor preparation, responses recorded from the internal rectus muscle. Stimulating electrodes in position F_1 (Fig 3) The curves are plots of the height of the testing response against the interval between conditioning and testing F shocks

1. (Expt 25-VI-34) Conditioning shock 120/100 stronger than the testing shock The heights of the conditioning and unconditioned testing responses were not constant, but varied between the limits indicated at the left of the curve, P_1 and P_2 It is to be noted that after a short period of facilitation a period of inhibition (extinction) developed

2 (Expt 9-I-35) Conditioning shock 60/100 of the testing shock P_1, height of the response to the conditioning shock, P_2, height of the response to the unconditioned testing shock It is to be noted that after an initial period of depression ending at the interval of 0 5 msec between shocks (cf Fig 9, 4), a short period of facilitation appeared which was followed by a period of inhibition

3 (Expt 9-I-35) Conditioning shock 120/100 of the testing shock The response to the conditioning shock had a height slightly larger than P_2 in curve 2 The unconditioned testing response had the height P_* The conditioning shock was followed by a period of unresponsiveness, attributable to refractoriness of the fibers that had been stimulated, afterwards there was a short period of facilitation and then a period of inhibition (from Lorente de Nó, 1936, Fig 1)

impulses meet in the motor nucleus a sufficiently dense volley of internuncial impulses with which to summate; a sufficiently dense internuncial volley in some cases is produced when the impulses initiated by the conditioning volley have crossed through one internuncial synapse; but in other cases the internuncial volleys are not dense enough until after two or three internuncial synapses have been crossed (cf. *Idem.*, 1938b, Fig. 1; 1938d, Fig. 3).

Similarly it may be explained why facilitation, although usually lasting

for 6–8 msec., sometimes lasts only 1–2 msec. (Fig. 8, *1*) and at other times considerably longer, even 80 msec. (Fig. 8, *6*). A convincing explanation can also be offered for the fact that in some preparations (Fig. 13) the phase of facilitation may be followed by a phase of depression of the testing response, which is comparable to the "extinction" described by Dusser de Barenne and McCulloch (1934, 1935) of the responses initiated in the cerebral cortex by electric shocks. Brief facilitation means that the internuncial activity was increased for a short period of time, while prolonged facilitation indicates that the internuncial activity was increased for a correspondingly long time. And the appearance of inhibition ("extinction") is explained by the fact that after a period of increased activity the internuncial chains temporarily cease conducting the impulses of the tonic excitatory background (Lorente de Nó, 1936).

Direct evidence of internuncial activity. Direct proof of the activity of the internuncial system would consist in a recording of the internuncial impulses when they are entering the motor nucleus. In an early report (*Idem.*, 1935*d*, Fig. 3, 8, 9) it was indicated that an electrode placed on the pathways which have synapses on the oculomotor nucleus could be used to detect the passage of internuncial impulses, and that delivery of a shock to the posterior longitudinal bundle resulted in the arrival at the level of the electrode of a first wave of negativity, attributable to the impulses directly created by the shock, and then of several other waves which were attributed to impulses that had crossed through internuncial synapses. Technical difficulties however, prevented a satisfactory analysis of the phenomenon.

A new attempt to record the internuncial impulses has been made after several investigators had reported encouraging results obtained with microelectrodes thrust into the brain (cf. especially the results of Renshaw, Forbes and Drury, 1938). It seems that the new experiments,* to be mentioned presently have afforded a satisfactory proof of the internuncial activity, and in addition have revealed significant data concerning the electric signs of the activity of somas of neurons. The records in Fig. 14–17 measure differences in electric potential between a micro-electrode placed near the active elements and a distant electrode located on inactive tissue, for example, the thalamus or the skull.

Distribution of bioelectric currents in volume conductors

This system of recording yields results quite different from those ordinarily obtained when a nerve surrounded by a dielectric is mounted on electrodes because, under these conditions, and in so far as the recording electrodes are concerned, the nerve acts approximately as a linear conductor of the currents of action, but the micro-electrode thrust into the brain-stem records differences in potential created by the spread of bioelectric currents in a volume conductor.

The problem of distribution of electric currents in a volume conductor was presented in a form suitable for the interpretation of neurophysiological problems by Helmholtz

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* Cf. comparable experiments of Dusser de Barenne and McCulloch (1939) and of Bishop and O'Leary (1936).

CENTRAL SYNAPTIC TRANSMISSION

(1853) and by Hermann (1879) * It follows from the theory of potential functions that whenever a nerve cell or fiber surrounded by a conducting medium becomes active and differences of electric potential are established between parts of the active element, electric currents appear in the medium in such a way that at any point outside the active element the potential† satisfies Laplace's equation No appreciable current will pass through a point distant from the active element, and therefore, when pitted against a distant electrode, a micro electrode will record a positive potential when it is located near the source of current, and a negative potential when located near the sink, the recorded potential being the greater the nearer the electrode is to the electromotive surface Thus, the sign of the recorded potential is meaningless unless the position of the micro electrode is accurately determined by subsequent histological analysis of the brain or by some other procedure

It is a consequence of the physical laws which determine the distribution of current in volume conductors that the form of the recorded potential wave sign of the conduction of the spike process of a nerve impulse will depend, among others, upon two conditions (i) the position of the micro electrode in relation to the active element, and (ii) the direction in which the impulse is travelling An example will bring out clearly the type of records that may be expected Assume a nerve with an end in the air and the rest of the nerve, including the other end, submerged in a conducting medium An impulse is initiated in the end in air and its passage along the nerve is recorded by three micro electrodes, each pitted against an electrode located at some distant point of the medium The first micro electrode (i) is placed on the nerve at the point of entrance into the medium, the second (ii) on the nerve, somewhere between the point of entrance into the medium and the submerged end, and the third (iii) at the submerged end

For a medium with the conductivity of the brain tissue and for lengths of nerve such as exist in the oculomotor preparation it can be predicted from the theory, and easily proved by experiment, that each of the 3 micro-electrodes will detect the existence of currents, as long as the nerve impulse occupies any part of the nerve, but the form of the recorded potential wave will be different for each micro electrode At electrode 1 the potential wave will be diphasic (negative, positive) because when the impulse reaches the medium the point of entrance of the nerve is the first to become a source, and since during the spike process have a negative sign until that point becomes a sink, the recorded potential will no further reversal of the current of the spike process ceases At electrode 2 the recorded wave will be triphasic (positive, negative, positive), indicating that that point of the nerve successively acts as a source, a sink and a source The relative size and duration of the three components of the triphasic wave will of course vary with the position of electrode 2 on the nerve For example, the first positive component will be very brief if electrode 2 is placed near the point of entrance of the nerve into the medium, or relatively very long if it is near the end, but all three phases will appear at any point of the nerve except as points 1 and 3 At electrode 3 the record will again have only two phases, a first positive phase while that point acts as a source, and a second negative phase while that point is drawing current from points of the nerve in which the recovery is more advanced, since the end of the nerve is the last point reached by the impulse, point 3 will act as a sink until the end of the spike process

A more recent, but not as rigorous presentation, may be found in Wilson, McLeod and Barker (1933), interesting model experiments have been described by Bishop (1937), cf too, O'Leary and Bishop (1939)

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* A more recent, but not as rigorous presentation, may be found in Wilson, McLeod and Barker (1933), interesting model experiments have been described by Bishop (1937), cf too, O'Leary and Bishop (1939)

† The word potential is used strictly in the sense defined in mathematical physics "Potentials" means values of a potential function, that in the discussion in the text is assumed to owe its existence to the presence of an electric current between parts of the active element (Fig 25, II) The discussion made in the text would, therefore, not directly apply to differences of potential due to other kinds of processes For example, it could not be directly applied to potentials generated by extracellular concentration gradients In the text it is assumed, because it is permissible to do so, that at least during the short interval of time after stimulation that is considered in this report, the recorded potentials are preponderantly due to changes in the electromotive force of the polarized membranes of the somas and the axons

Potentials recorded from the oculomotor nucleus and nerve

As in the case of the oculomotor nerve the length of the central segment of the nerve is short, the duration of the potential wave recorded from any point of the central segment of the nerve, or from the motor nucleus, should have approximately the duration of the spike potential as recorded from
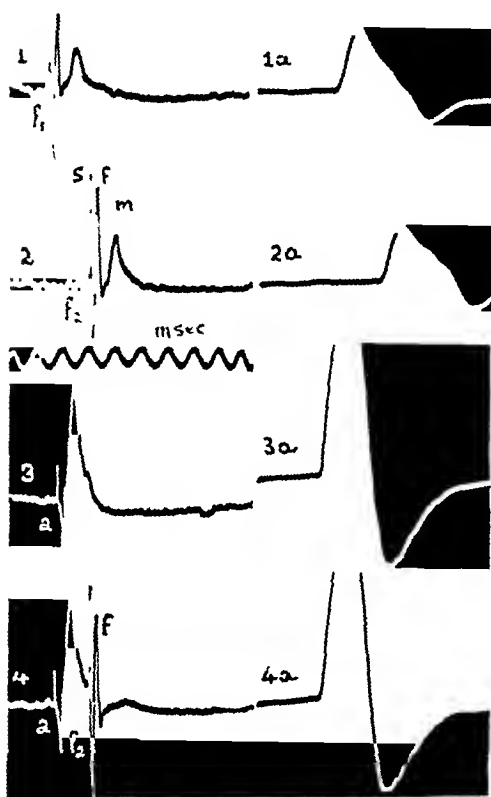
FIG. 14. Oculomotor preparation; responses recorded with two oscillograghs, from the motor nucleus (*1* to *4*) and from the internal rectus muscle (*1a* to *4a*). (Expt. 16-IV-39). Stimulating electrodes in position *F1* (Fig. 3). The responses from the motor nucleus were recorded with a micro-electrode about 50μ in diameter introduced into the nucleus at the point where the motor axons collect in a dense bundle to form the motor nerve. The indifferent electrode was placed in the oral and dorsal portion of the thalamus, *i.e.*, in a part of the brain that is not reached by the *f* impulses.

1 and *2*. Potentials developed by *F* shocks. *s*, shock artefact; *f*, potential of the *F* impulses; *m*, potential of the motoneurons; *1a* and *2a*, the responses of the internal rectus muscle.

3. Potential developed by the entrance of a maximal antidromic volley *a* into the motor nucleus. *3a*, response of the internal rectus muscle.

4. The *a* and *f₂* shocks are delivered in rapid succession. The antidromic volley, by creating refractoriness in the motoneurons, obliterates the largest portion of the *m* wave. *4a*, response of the internal rectus muscle. The negative deflection in *3* measures well over 6 millivolts. Time in msec. between records *2* and *3*.

nerve with the ordinary technique, plus the conduction time along the nerve. Contrary to this expectation it has been found that a micro-electrode may record potentials of much longer duration. Therefore, in addition to the "travelling" process which gives rise to the ordinary spike potential, it must be assumed, that there exists some other "standing" process, which results in enduring differences of potential between different parts of the motoneuron, presumably between the soma and the initial segment of the axon. This significant result will be presented here in some detail.

Records *1* to *4* in Fig. 14 were obtained with the micro-electrode near the point where the motor axons collect into a dense fasciculus to leave the nucleus. Thus, *in so far as the motoneurons are concerned*, the records may be said to indicate the existence of currents that are leaving or entering the initial segment of the motor axons. In the position in which it

was placed, the micro-electrode also was sufficiently near the posterior longitudinal bundle to record differences of potential created by the passage of impulses through this bundle. A shock was delivered through electrodes F

(Fig. 3, $F1$) with the result that the posterior longitudinal bundle conducted a volley of impulses (f) which resulted in discharges of motoneurons (Fig. 14, $1a$, $2a$). The micro-electrode recorded besides a sharp shock artefact (s, Fig. 14, 2), the impulses conducted by the posterior longitudinal bundle (f) and then the motor impulses (m).

The f potential wave should have shown three phases, because the f impulses are conducted past the recording micro-electrode. The two first phases (positive, negative) are clearly visible, but the third phase is obliterated chiefly by the potential wave caused by the motor impulses m.

That the m wave was due to the discharge of motoneurons is easily proved. Delivery of a maximal antidromic shock (Fig. 14, $3a$) to the oculomotor nerve outside the brain stem resulted in a similar wave having of course a large initial phase of positivity. When shortly afterwards the F shock was delivered (Fig. 14, 4), the m wave (Fig. 14, 2) failed to appear, owing to the fact that the motoneurons were just beginning to recover from absolute refractoriness.*

FIG 15 From the same experiment of Fig 14, but the amplification for records 1, 2 and 3 higher than in Fig 14

1 Discharge of impulses of the tonic labyrinthine innervation, which, owing to the low amplification used, is not visible in record $1a$

2 Three F shocks in succession, indicated by arrows, like f_1 and f_2 in Fig 14, set up a synaptic response of motoneurons, $2a$, responses of the internal rectus muscle

3 A maximal antidromic shock creates a volley of impulses entering into the motoneurons Note that in 2 as well as in 3 the refractoriness of the motoneurons produces a silent period of the tonic discharge Time, 200 cycles above record $1a$

The records in Fig. 15 were obtained at higher amplification and reveal significant facts not clearly shown by the records in Fig. 14. Record 1 in Fig. 15, obtained without stimulation, shows a succession of small irregular spikes which obviously belong to those motor impulses that were maintaining the labyrinthine tonus of the eye muscles.

* It will be noted by comparing records $3a$ and $4a$ in Fig 14 that a few motoneurons did respond, thus preventing satisfactory analysis of the small negative deflection which in record 4 follows the sharp negative deflection of f This negative deflection in part must be attributed to the entrance of the f impulses in presynaptic fibers and synaptic knobs

Record 2 was obtained by delivery of three approximately equal F shocks at intervals at which facilitation is usually observed. It will be noted in record 2a that although the response to the first shock was small, the responses to the second and third were large; indeed, since all three responses make out a potential larger than that obtained after delivery of a maximal antidromic shock, there can be no doubt that all the motoneurons had fired and that at least the third response in 2a included motoneurons that had already taken part in one of the previous responses.

Finally record 3 was obtained by delivery of a maximal (antidromic) shock to the motor nerve. Comparison of records 2 and 3 shows that the micro-electrode, besides recording sharp negative deflections, which approximately signal the passage of the *travelling* spike process, also recorded an enduring positive deflection, which undoubtedly indicates the existence of a *standing* difference of potential. As in records 2 and 3 all the motoneurons had responded, the final positive phase is approximately equal in both cases, the slight difference being attributable to the fact that in the case of record 2 the motoneurons fired in successive volleys.

The interpretation of the prolonged positive phase is not an easy matter. As already pointed out, the fact that the micro-electrode recorded a change of potential in the positive direction indicates only that it was located near a source of current and that some other part of the active element was a sink for that current. That during the third phase of the recorded wave the initial segments of the motor axons are sources of current is beyond doubt, but concerning the position of the sinks the evidence is not so conclusive. When the micro-electrode is moved peripherally along the central traject of the motor nerve, the third phase in question rapidly diminishes in size, but without reversing its polarity. This fact indicates that the main sinks are not located in the more peripheral parts of the axon. Therefore, they must be located in the soma of the motoneurons, and in fact, in the case of the hypoglossus nucleus, in experiments conducted especially for that purpose, the potential differences are by far greatest within the motor nucleus and in the zone where the axons collect to leave the nucleus. Furthermore, records have been obtained with the micro-electrode placed in the nucleus in which the potential wave, initiated by the entrance of antidromically conducted impulses, had only a first positive and a second negative wave, which ended at about the same time as the third or positive phase of the wave recorded from the initial part of the axons. However, the conditions for recording potentials are often unfavorable, because in the act of introducing the rather coarse micro-electrode (about 50μ in diameter) into the motor nucleus, the neighboring motoneurons are compressed and finally killed (cf. O'Leary and Bishop, 1939) before the micro-electrode is sufficiently near to any of them to be affected by the currents, that are entering the soma of motoneurons in a much higher degree than by the currents which are leaving neighboring axons. Thus, the conclusion derived from the experiments in question is

not definitive It may be stated in the following sentences The evidence available at present indicates that after conduction of an impulse initiated by synaptic or antidromic stimulation, the soma of the motoneurons remains negative in relation to the initial part of the axon, i e draws current from the latter during considerable periods of time, according to records such as those in Fig 15 for 25–30 msec , or even longer A later reversal of current has not been observed, but since the amplification was never high, a late reversal might have been overlooked

Apparently, then, the temporal course of the depolarization and repolarization taking place during activity is not the same for the soma as for the axon of the motoneuron, as the depolarization in the former lasts for a longer period of time than the depolarization of the axon during the familiar spike process

Comparison of potential differences in motoneuron with nerve potentials An important peculiarity of the standing potential difference at the motoneuron i e of the relative negativity of the soma, is that it is a sign of depressed synaptic excitability It will be noted in Fig 15, 2 and 3 that during the third phase of the potential wave the tonic discharge of motor impulses visible in Fig 15, 1 was first completely stopped and then progressively reestablished as the potential difference diminished This phenomenon is analogous to the familiar "silent periods" in reflex discharges (Hoffmann, 1920) which at present are generally attributed to subnormal excitability of the elements involved (cf Gasser, 1937a, p 200) Furthermore, it will be noted that the recovery curve of the motoneurons (Fig 6, 2) parallels in a striking manner the third phase of the potential wave in Fig 15, 2 and 3 *

In this respect the enduring negativity of the motoneurons is not directly comparable to the negative after potential of nerve, because during the latter the excitability of the nerve is supernormal (cf Gasser, 1937a, p 174) There is still another reason that prevents a direct analogy The after potentials indicate the existence of differences of electric potential between points of the nerve which have been traversed by the impulse and a killed zone of the nerve which has not conducted an all or-nothing disturbance, but in the case of the motoneuron the recorded differences of potential are established between two parts of the element that have conducted the same impulse That after conduction the active soma of the motoneuron becomes negative

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* The electrical signs of the activity of the oculomotor neurons reported here are not directly comparable with the electrical signs of the activity of spinal motoneurons described by Eccles and Pritchard (1937) and Eccles (1939) and also mentioned by Gasser (in this Symposium) The differences in the recorded potentials however, might be attributable to differences in the recording techniques that have been used On the other hand the recovery cycle of ocular motoneurons (Fig 6, 2) appears to be different from that reported for spinal motoneurons The differences are great when comparison is made on the basis of the description of Eccles and Pritchard but become insignificant when the comparison is based on the observations of Gasser As the present author has had no experience with spinal motoneurons detailed discussion of these points would be unprofitable

in relation to the active axon does not indicate what either of them would show when pitted against a part of the axon which by suitable injury has been deprived of the ability to conduct impulses. It appears, therefore, that in the present state of knowledge it would be premature to attempt a close comparison of the enduring phase of relative negativity of the soma of the motoneuron with potentials measured in nerve by means of ordinary techniques (cf. the more detailed discussion at the end of this report).

Potentials recorded during internuncial activity. Electrical signs of inter-
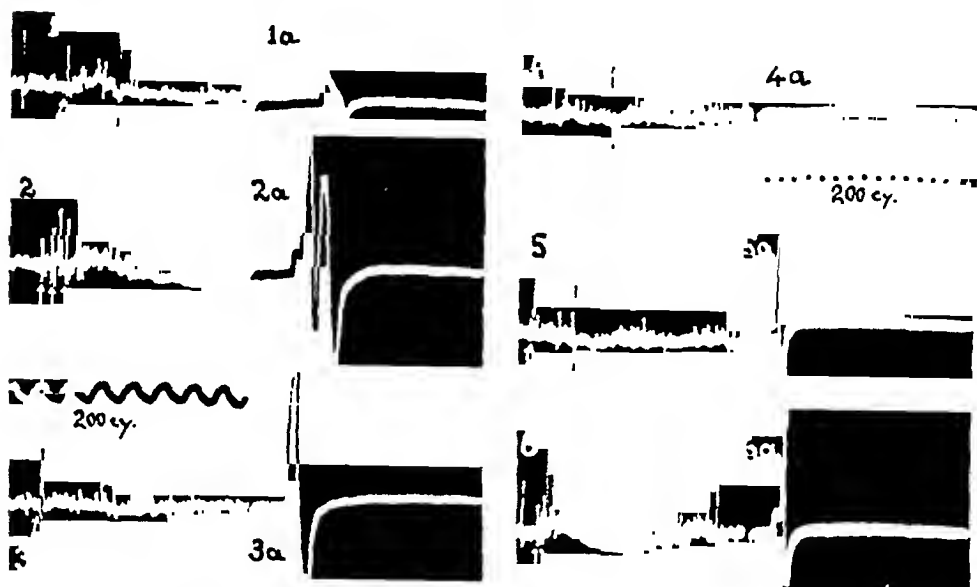
Fig. 16. From the same experiment of Figs. 14 and 15. *1* to *6*, responses recorded from the internuncial nuclei with a microelectrode introduced at about the center of the nucleus labelled *M.b.* in Fig. 3.

1. One *F* shock, marked by the arrow, sets up a moderate increase of the internuncial discharge. *1a*, response of the motoneurons recorded from the internal rectus muscle.

2. Three *F* shocks, marked by the arrows, like those used in Fig. 15, *2*, set up a marked increase of the internuncial activity. *2a*, response of the motoneurons.

3. A maximal antidromic shock, marked by the arrow, does not alter the internuncial activity. *3a*, response of the internal rectus muscle. Time for records *1* to *3*, 200 cycles between records *2* and *3*.

4. No stimulation. The sharp spikes belong to impulses of the tonic internuncial discharge.

5. A maximal antidromic shock, marked by the arrow, fails to alter the internuncial activity.

6. Two *F* shocks, marked by arrows, cause an increase of the internuncial discharge, followed by a silent period; the discharge is reëstablished when the internuncial elements recover from refractoriness. Time, 200 cycles between records *4a* and *5a*.

nuncial activity can easily be recorded; nothing else is needed but to introduce the micro-electrode into a pool of interneurons. For example, the records in Figs. 16 and 17 were obtained with the tip of the micro-electrode in-

troduced into a pool of interneurons, oral, lateral and ventral, to the oculomotor nucleus, *i.e.* about at the center of the pool labelled *M.b.* in Fig. 3 (cf. 1938d, Fig. 1. *H*).

Records obtained at low sweep speed (4) in the absence of stimulation show signs of persistent activity, because they contain an irregular series of spikes that signal the passage of impulses which are either arriving at or leaving the internuncial pool. The internuncial activity was of course not changed when maximal antidromic shocks were delivered to the oculomotor nerve.[*] There appeared (cf. the arrows in 3 and 5, Fig. 16) a sharp triphasic
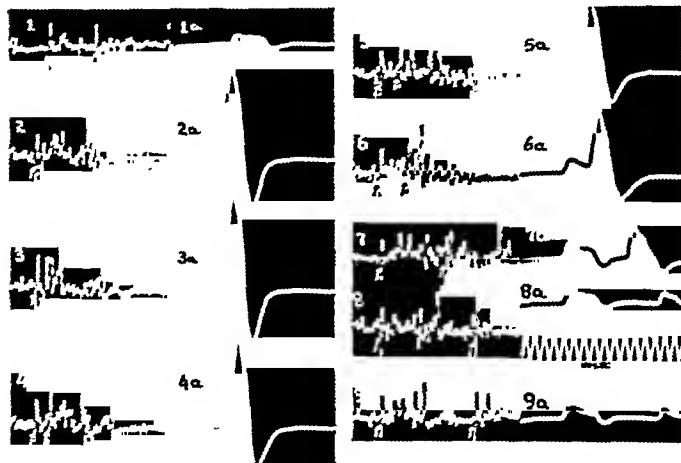
FIG. 17. From the same experiment of Fig. 14-16. *1* to *9*, responses of the internuncial nucleus, as in Fig. 16. *1a* to *9a*, responses of the internal rectus muscle. Time, 1000 cycles between records *8a* and *9a*. The records illustrate the relation between internuncial activity and facilitation of the motoneurons. Further details in text.

spike, a sign that the motor impulses on their way to the motor nucleus had passed at some distance from the micro-electrode, but the internuncial activity was not altered.

In contrast, an *F* shock to the posterior longitudinal bundle, which resulted in a small response of the motor nucleus (Fig. 16, *1a*), caused an ap-

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[*] *Records such as 3 in Fig. 14 and 3 and 5 in Fig. 16 conclusively prove that conduction across the synapses on the motoneurons is irreversible. Synchronous activation of all the motoneurons failed to set up impulses in fibers having synapses on them. There is no doubt that if the synapses had transmitted in the antidromic direction, the new impulses would have been recorded.*

preciable increase of the internuncial activity (Fig. 16, *1*). The increase was much more pronounced when three *F* shocks were delivered in succession. The motor response (Fig. 16, *2a* was then as strong as in the case of Fig. 15, *2a*, and, as had been predicted, there appeared immediately after the *F* shock a spectacular increase of the number of internuncial impulses passing near the micro-electrode (Fig. 16, *2*). A remarkable peculiarity of the phenomenon was that the temporary increase was followed by a marked, also temporary, decrease of the internuncial discharges. When recorded at slow sweep speed (Fig. 16, *6*) the decrease of activity following the initial increase again had the appearance of a "silent period" with a similar electrical sign and with only slightly longer duration than the silent period in Fig. 15, *2* and *3*. One would, therefore, be inclined to conclude that, following activity, the internuncial axons for a certain period of time also remain electropositive in relation to their somas.

The suspected close correlation between the changes of activity of the internuncial pools and the excitability of the motor nucleus was found. The period of facilitation of the *F* responses is well illustrated in Fig. 17. A testing shock created a mild increase of internuncial activity (*1*) and initiated a small motor response (*1a*); but when this shock was preceded by another smaller shock (cf. Fig. 14, *f₁*, *f₂*) both the size of the motor response (*2a*) and the degree of the internuncial activity (*2*) increased enormously. Upon examining the records in Fig. 17, it will be noted that when the testing shock was delivered during the period of increased internuncial discharge (*2–7*), the testing response was facilitated (*2a–7a*); when, however, the testing *F* shock was delivered after subsidence of the increase of internuncial activity (*8, 9*), the testing response did not show any facilitation (*8a*), but rather a depression (*9a*). In this experiment slight spontaneous variations of excitability of the preparation (cf. the conditioning responses in records *4a, 5a, 6a, 7a*) prevented an accurate study of extinction after a single conditioning shock, but in other experiments in which the observations could be properly made, it was established that during the "silent period" of internuncial activity (Fig. 16, *6*) there was an inhibition (extinction) of the testing response exactly as it had been concluded (1936) from evidence not so direct.

It is an immediate consequence of the anatomy of the internuncial system, and therefore an incontrovertible fact, that at least a large number of the internuncial impulses recorded in Fig. 16 and 17 entered the motor nucleus and were delivered to the motoneurons. Therefore, the experiment illustrated in Fig. 16 and 17 constitutes final proof that a shock to the posterior longitudinal bundle initiates a volley of impulses, which not only is delivered to motoneurons and eventually causes them to respond, but also to interneurons and stimulates a number of them to discharge new impulses; this internuncial discharge increases the already existing internuncial activity during a certain period of time. While that activity is increased, the motoneurons are submitted to a powerful bombardment, which lowers their threshold and eventually may result in new motor discharges (cf.

1938d, Fig. 3). The increase of internuncial activity may subside gradually, in which case nothing but a decrease and final disappearance of facilitation are observed; but it may also happen that the increase in internuncial activity is followed by a "silent period" of the internuncial chains, in which case the facilitation of motoneurons is followed by inhibition.

III. General Interpretations[*]

In view of the evidence now available there can be no doubt that in a first approximation it is permissible to explain the activity of the nervous system in terms of the activity of chains of neurons and the detonator actions of the nerve impulses, i.e., in terms of all the anatomical elements that the nervous system contains and of the nerve impulses that they produce or carry. Moreover, explanations of this type are forcibly demanded by a considerable body of established facts. When working out the theories in detail it may, and it probably will be found, that consideration of the activity of the chains of neurons, the properties of synaptic transmission and the changes in threshold during the recovery cycle of neurons which have been active—and these were the only factors considered in detail in a previous report (1938d)—is not sufficient to explain in a satisfactory manner all the known facts. Then, it will be necessary to introduce corrections into the theoretical arguments, taking into account the possible development of of a second phase of summation in subliminally stimulated neurons, chemical changes or changes of ionic concentration in the environment, possible influence of the action currents of active upon inactive elements (cf. Adrian, 1932; Jasper and Monnier, 1938; Eccles and O'Connor, 1938; Katz and Schmitt, 1939), and perhaps other as yet unknown or unsuspected processes (cf. 1938d, p. 240, footnote.) In certain cases the corrections may prove to be of paramount importance because the neuron works at threshold, i.e. it fires as soon as its threshold is reached, and the transmission through the chains of neurons is a conditional event; but still the basic assumption in the interpretation of the activity of the nervous system must be made in terms of the activity of the internuncial system. It is obvious that in the central nervous system internuncial bombardment is the primary factor which creates all the others. In its turn, however, the internuncial bombardment will be modified by the changes that it creates.

For example, it is known that under proper environmental conditions a nerve cell may become rhythmically active (cf. Bronk, in this Symposium). Let it be assumed as Barron and Matthews (1938) have done, that the environmental change required for initiating rhythmic activity of neurons takes place in the central nervous system during normal function. Then it would be possible to single out any interneuron and consider it a pacemaker, but as soon as this had been done, it would have to be realized that the pacemaker could not keep on driving other neurons by virtue of its own auto-

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[*] Cf. the similar views held by Dusser de Barenne and McCulloch, 1939.

matism, because if the pacemaker forces other neurons to discharge, impulses will be delivered back to it through the internuncial chains, with the result that the driving pacemaker will then be driven by its fellow interneurons!

Theories of synaptic transmission

The experiments mentioned in this report have revealed data that have significant bearing on the problem of synaptic transmission of impulses to motoneurons. The information obtained defines the temporal course of transmission, establishes certain relations between the arrival of impulses at synapses and changes of electric and synaptic threshold of the neurons, etc. Thus it may be said that the experiments herein reported help to state the problem of synaptic transmission to motoneurons in precise terms, but they are insufficient to solve the problem.

Since it has been demonstrated that the soma of the motoneuron, whereupon the synapses are located, is electrically excitable (1935f; Barron and Matthews, 1938) and it may be taken for granted that the nerve impulse at its arrival at the synapses creates there differences of potential, it is permissible, even logical, to assume that the soma of the motoneuron is stimulated to discharge an impulse by these differences of potential. One might go so far as to believe that the available evidence is adequate proof of the essentially electrical nature of the process of transmission, but it must be realized that if the assumption were made—and such an assumption has indeed been made (Feldberg and Vartiainen, 1934)—that at the synapse a chemical agent is released in such a way that its production, action and inactivation follow a temporal course more or less comparable to that of the action current of the nerve impulse,—then, of course, the experimental facts would be compatible with chemical transmission (cf. 1935f, p. 69); and, if postulates such as those suggested by Barron and Matthews are made, then, the differences between chemical and electrical transmission may be reduced to almost pure formality (cf. Barron and Matthews, 1938, p. 315).

The difficulty consists in ascertaining whether the assumptions are supported by factual evidence, and, if this is the case, how far they may be developed; the difficulty is indeed not negligible, because it will happen that even the "facts" must be rechecked. For example, Feldberg and Vartiainen (1934) believed they had established beyond doubt that acetylcholine is specifically released at the ganglionic synapses during the act of transmission. Much was based on their conclusions and a finished theory was developed (cf. Dale, 1938), but upon reëxamination of the factual material the present author found (1938a) that acetylcholine is neither a specific product nor released with the regularity and temporal course that had been assumed.* The new findings are not entirely incompatible with chemical

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* The validity of my experiments with perfusion of the superior cervical and nodosum ganglia (1938a) has been questioned by MacIntosh (1938-9). MacIntosh denies almost every statement that I made; but since Lissák (1939), in agreement with my results, re-

transmission, but if transmission by the synaptic release of acetylcholine should be maintained, important modifications would have to be introduced in the original theory. The need of revision is also shown by other circumstances, of which a few will be mentioned here. Chemical transmission with essentially different characteristics was postulated by Rosenblueth (1934) and strongly supported by Forbes (1934); but more recently Cannon and Rosenblueth (1937, and later papers) have formulated the chemical theory in a manner that deviates from the views formerly held by Rosenblueth (1934) and also from the views held by the London school. Brown (1937) and Eccles (1937) from their discussions of the same factual material, arrived at opposite points of view. Thus, Eccles concluded that acetylcholine is not the synaptic transmitter to ganglion cells, although it might play some rôle, among other things, on lowering their thresholds. This view, which is untenable on the basis of the postulates set forth by Dale (1938), is nevertheless compatible with the observations of the present author (1938a), with the experimental results of Bronk, Tower, Solandt and Larrabee (1938), and with facts presented by Bronk in this Symposium. Again, in order to assume that acetylcholine could be a facilitative agent, assumptions would have to be made regarding the inactivation of acetylcholine which would be accepted by some investigators and rejected by others.

No valuable purpose would be served by making further quotations from the extensive literature that has accumulated in recent years. A number of conflicting hypotheses have been made and others will undoubtedly be made. While the discussions go on, the following statement seems reasonable that *the action currents of nerve impulses arriving at the synapses may prove not to be the agents for synaptic transmission, but everything happens as if they were*. How this sentence should be understood will appear in the discussion that follows.

A model of synaptic transmission

In the study of problems not easily accessible to direct experimentation it is often useful to examine the phenomena, which under similar conditions, appear in artificial models. In neurophysiology there is scarcely any need of justifying the use of models because the well-known core-conductor model of Hermann, the membrane model of Labes and the iron wire model of Lillie have played important rôles in advancing and diffusing knowledge. Models, it is true, do not solve problems, but they clarify concepts and suggest working hypotheses. For these reasons attention is now directed to a model of synaptic transmission.

One of the most important contributions ever made to neurophysiology was the publication by Wedensky (1903) of his detailed study of the properties of a block of conduction in nerve. Wedensky, after comparing the

ports release of acetylcholine by the vagal ganglia and this confirms my conclusion that acetylcholine is not a specific synaptic product, I may perhaps hope that some others of my conclusions were also correct.

properties of the block with those of the normal and the curarized neuro-muscular junction, reached the conclusion that the block imitated conditions found at the synaptic junction. Wedensky's block is the synaptic model that will be studied here.

Observations of Wedensky. Wedensky and his co-workers used a large number of blocking agents (cocaine, phenol, heat, pressure, galvanic polarization, intense faradization, etc.). While differences were found to exist between the various blocks,* properties were established common to practically all the blocks. For the purpose of the following analysis of the block, the three most relevant properties are: (i) A partial block can transmit impulses at low frequency, but fails to transmit impulses at high frequency. This phenomenon is usually known under the name of Wedensky inhibition. (ii) After the blocking agent is applied to the nerve, the threshold of the treated segment is found to rise progressively, while the threshold of the segment below the block becomes lower than it was before. Entrance of impulses into the treated zone produces in it an additional enduring rise in threshold. (iii) A block that has become complete, either because the blocking agent has acted during a sufficiently long time or because impulses entering into it have strengthened the previous partial block, fails to conduct impulses; but while the impulses are stopped at the upper margin of the block, the threshold of the nerve below the block is lowered, the lowering of threshold being a cumulative and enduring process (cf. Wedensky, 1903, p. 65). This phenomenon will henceforth be called "Wedensky facilitation" or "facilitation across the block."

From the extensive literature dealing with Wedensky facilitation, inhibition and concomitant phenomena, only a few papers will be mentioned here. First are the contributions of Samojloff (1925) and Woronzow (1924, 1931) who, with string galvanometer, analyzed in greater detail and with greater precision than Wedensky (cf. Wedensky, 1903, p. 92) the electrical signs of the modifications that take place at the block; and then mention will be made of two papers by Hodgkin (1937a, b) that brought forth an important advance in knowledge.

Temporal course of the facilitation across the block; initial phase. Hodgkin established the relationship between the temporal course of the electrical sign of the blocked impulse and the temporal course of facilitation. One recording electrode at or shortly below the block, when pitted against another electrode also below, but at considerable distance from the block, records an electronegative variation when the facilitating impulse is stopped at the block. The recorded difference of potential is one that might be expected from electrotonic conduction through the block of the electric sign of the blocked impulse. The important fact is that the lowering of threshold at points below the block quantitatively follows the course of the recorded potential difference. In Hodgkin's observations the potential recorded below

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* A remarkable study of blocking agents has recently been made by Bishop (1934).

the block usually included a sharp spike-like deflection only, but in some cases it also showed a tail, which Hodgkin interpreted as being attributable to the negative after-potential of the blocked impulse
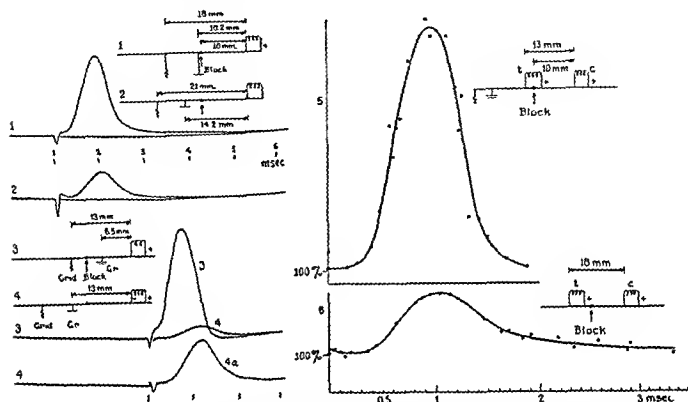
FIG 18 Facilitation across a Wedensky block in the sciatic nerve of a green frog (Expt 4-VIII-37) The block was created by applying to the nerve a cotton thread moistened with 0 75 per cent cocaine hydrochloride, at the point marked with *Block* on the nerve Distances are measured from the cathode of the conditioning coil

On the left side potentials recorded with the ground and grid electrodes placed at the points of the nerve, indicated in the diagrams with the same numeral as the records, after delivery of a maximal *A* shock through the conditioning electrodes

1 and *2* Potentials recorded below the block, the same amplification for both records, note the decrement and change of shape suffered by the spike-like potential (detonator negativity) during its transmission through four mm of nerve, and note also that the detonator negativity is followed by a residual negativity relatively larger in *2* than in *1*

3 The spike recorded above the block at an amplification lower than for records *1* and *2*

4 The spike like potential recorded below the block at the same amplification as for *3*

4a, the potential of *4* at higher amplification On the right side, facilitation of the response to a submaximal shock produced by an impulse that is stopped at the block Conditioning and testing electrodes in the positions indicated in the records Abscissae, intervals between shocks in msec Ordinates, height of the conditioned response, 100 per cent, height of the unconditioned response Note that the ordinates scale in the upper curve is smaller than that of the lower curve Compare shape of the facilitation curves with the potentials on the left side and note that in the lower curve the potential produced by surge to ground (shock artefact) also lowered the threshold of the nerve, apparently in a similar manner as the later arriving spike-like potential

There is not much that can be added to the description of Hodgkin (1937a, b) of the initial phase of the phenomenon, except perhaps to present records (Fig. 21) which show in greater detail than those of Hodgkin the modifications suffered by the potential wave while it is being transmitted electrotonically through the block and points below the block. Also, it is
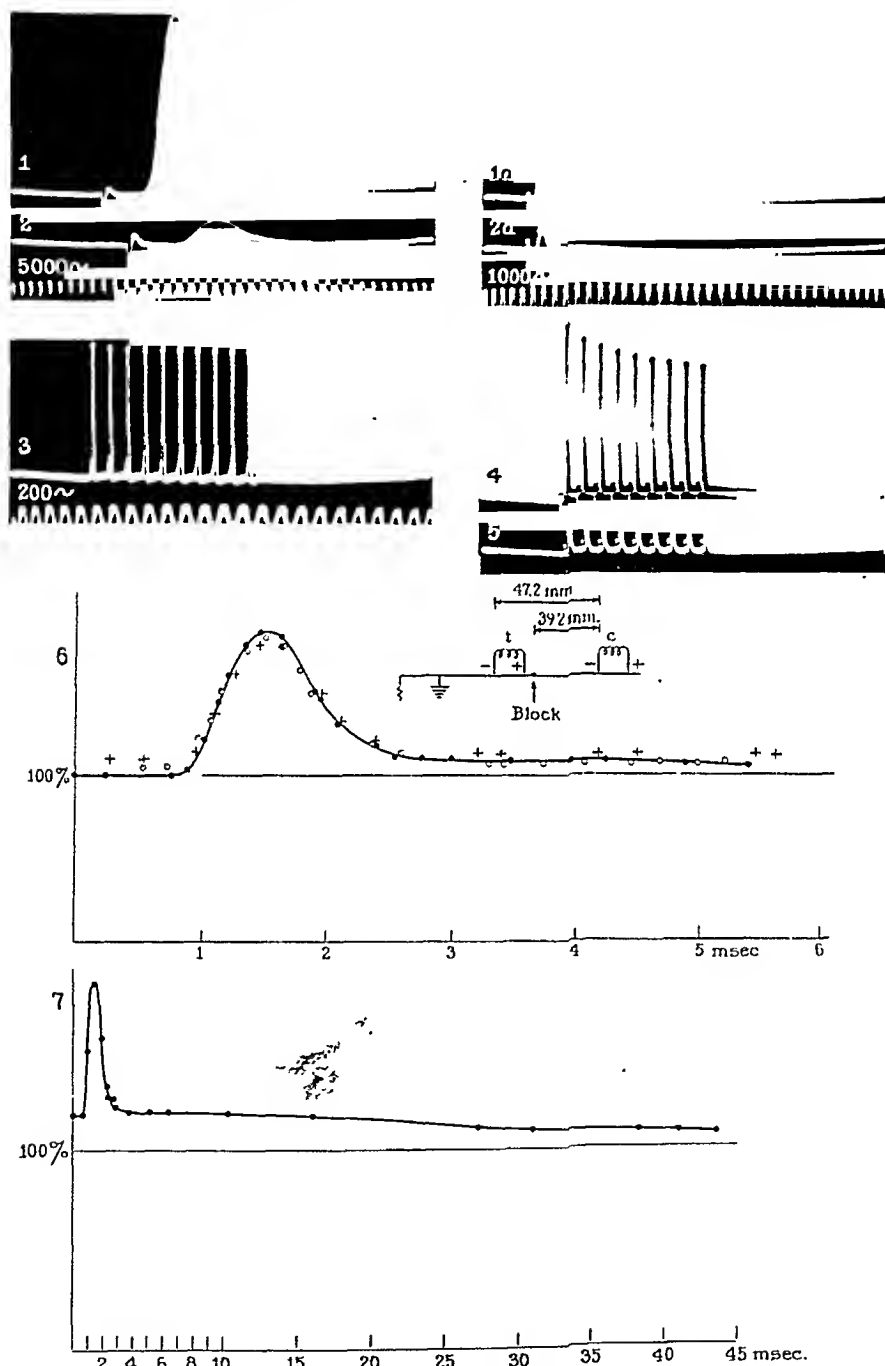
FIG. 19. (See next page for legend.)

pertinent to publish "facilitation" curves obtained with the testing cathode at different distance below the block (Fig. 18). After one impulse the electrical signs and the changes in threshold below the block, following the short interval of time considered by Hodgkin, are not great (Fig. 19, *6*), but when the block is conditioned by a train of impulses, the late phases of the phenomenon, which obviously played a significant rôle in Wedensky's experiments are very prominent (Fig. 19, *7*, and 20).

Facilitation by a train of impulses. Disregarding the existence of a demarcation current through the block and neighboring points of the nerve, a general description of the phenomena may be made in a few sentences. Arrival at the block of a train of impulses creates in the block, besides rapid changes similar to those observed after single impulses, a certain progressive change which, during and after arrival of the train, results in the creation of differences of electric potential between the block and points above and below it. When the currents are recorded with an electrode at or below, but near the block, and another electrode far below the block, the currents reveal themselves as a sequence of potentials resembling the familiar spike negative after-potential positive after-potential sequence. Negativity below the block indicates the existence of a lowered threshold, and positivity, the existence of a raised threshold, in comparison with the threshold before the conditioning. In this respect the relationship between excitability and electrical signs follows the rule that is given by Gasser (1937*a, b*; cf. also, in this Symposium) for true after-potentials, *i.e.* for the after-potentials recorded with a nerve that has conducted all-or-nothing disturbances. The absolute value, temporal course, and relative size of the three components of the potential sequence recorded below the block change progressively when the upper electrode is approached to the fixed electrode far below the block.

FIG. 19. Facilitation across the block produced by a train of impulses (Expt. 6-VIII-37). Bullfrog sciatic, cocaine block at 39.2 mm. from the conditioning cathode. Testing electrodes as indicated in the diagram, (*t*). Recording electrodes for the impulses initiated at the testing cathode also indicated in the diagram.

1. Potential recorded at the testing anode, used as recording ground and pitted against the recording grid, after delivery of a maximal *A* shock through the condition electrodes (*c*). *1a.* The same at a slow time line.

2. and *2a.* Potentials recorded at the testing anode at the same amplification as for records *1* and *1a.*

3. A train of ten impulses recorded with the ground electrode above the block, 10 mm. from the conditioning cathode. Note that all the spikes are approximately equally high. Much lower amplification than for records *1* and *2.*

4. and *5.* The train of impulses recorded at and below the block, under the same conditions as for records *1* and *2.* Note the residual negativity and the drop in height of the spike-like potentials.

6. Facilitation curves, ● after one impulse, ○ after two impulses, × after five impulses of the train reproduced in *3, 4, 5.* Note the residual facilitation that corresponds to the residual negativity.

7. Facilitation after ten impulses. Note the long lasting effect of the residual negativity.
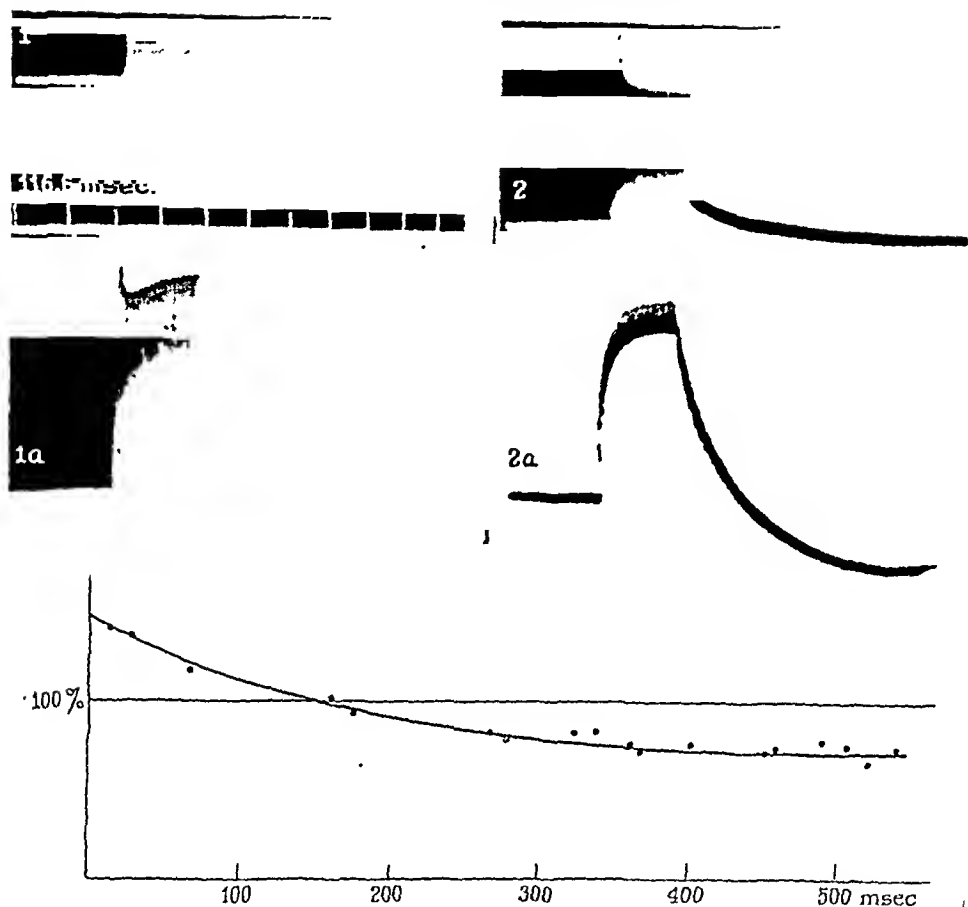
Fig. 20. Facilitation across a cocaine block. Bullfrog sciatic (Expt. 26-X-37). Conditioning by a train of impulses produced by shocks third of maximal A strength at the rate of 225 per second. When the train was recorded 11 mm. above the center of the block the spikes kept constant height, but when recorded at 6 mm. above the block they progressively diminished in height, thus indicating that cocaine had spread up to that point. Testing anode 2.5 mm. above center of the block (cf. Fig. 21, Block). Testing cathode 1.5 mm. below center of block.

1. Potentials recorded at the testing cathode connected as ground electrode and pitted against a grid electrode 29 mm. below the center of the block.

2. Potentials recorded at the testing anode. Same amplification as for 1.

1a and 2a. The potentials of 1 and 2 recorded at five and a half times higher amplification. Note that in 1 the cumulative residual negativity is much larger in relation to the spike-like potential than in 2, and note also that the residual negativity goes over to positivity in 2a about 100 msec. earlier than in 1a. Below, facilitation curve obtained after a train of impulses equal to those used for obtaining records 1 and 2. During the period of residual negativity at the testing cathode (1a) the threshold of the nerve was lower than the resting threshold, but during the period of positivity the threshold was higher and, therefore, the response smaller than when unconditioned.

There is no need of describing in detail the facilitation curves in Fig. 18, 19 and 20; it is sufficient to mention that the conduction block was obtained with 0.5 to 0.75 per cent cocaine hydrochloride, and to emphasize the similarity between the temporal courses of the changes in threshold and the recorded potentials. But a study of the recorded potentials will be made in some detail in order to compare phenomena at the block with phenomena at the synapse

Spike-like potentials below the block. In Fig. 21 only the spike-like potentials are included. There are in this figure two sets of records. Those

FIG 21 Cocaine block, bullfrog sciatic (Expt 26-X-37) Potentials recorded at different points of the nerve after delivery of a shock about one third of the maximal *A* strength Position of the stimulating and recording electrodes indicated in the diagram at the right The stimulating electrodes were kept at a fixed position as was the grid electrode, at point *11*, 60 mm from the cathode, for the records traced with thick lines, and at point *7* for the records traced with thin lines The recording ground electrode was successively placed at points *1* to *10* at the distances indicated in mm on the diagram The amplification was maintained constant, except that for records 9 and *10*, two different amplifications were used, the smaller being the same as for the other records For record 6 the shock strength was inadvertently doubled, which produced a height of response about twice the height of the response that would have been obtained with the shock used for the other records Note that the spike-like potential is decrementally transmitted through the block and below the block with the same apparent speed as the nerve impulse is conducted above the block The initial deflection in record *1*, immediately before the spike is due to electrotonic conduction of the stimulating shock
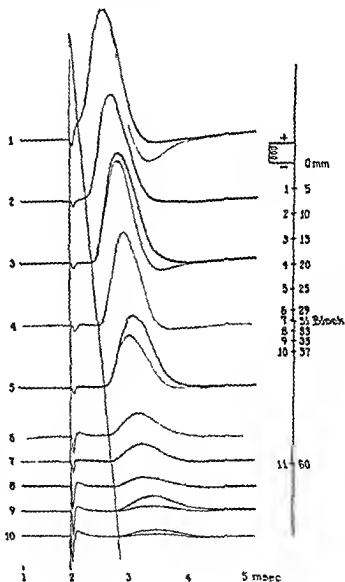
traced with thick lines were obtained with the fixed electrode on point *11* of the nerve, *i.e.* 29 mm below the center of the block, while those traced with thin lines were obtained with the fixed electrode on point *7*, i.e. at the center of the blocked segment. Therefore, records *1, 3, 4, 5* and 6 (thin lines) measure potential drops caused by currents at segments of the nerve above the block and at the upper margin of the block, while the other records (thick lines) include those potential drops algebraically summated with drops attributable to currents established between the block and points of the nerve below it.

An important fact is illustrated by records *1* to *6* (thin lines). Record *1* is diphasic, although the second phase is much smaller than the first one. The diphasicity is less marked in records *3* and *4*, and although very small, is still present in record *5;* but there is no sign of diphasicity in record *6.* Cocaine poisoning diminishes the conduction rate of the nerve impulse; therefore, owing to the lack of uniformity of the block, a progressive decrement of the rate of conduction took place when the impulse approached the block. This decrement, however, is not sufficient to explain the monophasicity of record *6.* When both recording electrodes are in contact with points of the nerve through which transmission takes place, the only case in which the recorded difference of potential does not cross the base line and change its sign is when the wave of negativity reaches the second electrode with sufficiently subnormal height (Hermann, 1878*a*, *b*). Where the decremental conduction began cannot be determined with accuracy because the spike included impulses in a number of fibers; but for the purpose of the present analysis, it is sufficient to establish the existence of the decrement at the margin of the block. As an important consequence of the decrement when the impulses reached the margin of the block, a steep potential gradient was created there—*e.g.* record *6* was obtained with the recording electrodes 2 mm. apart—with the remarkable peculiarity that the potential gradient had approximately the duration of the spike process as recorded at untreated points of the nerve and vanished without reversing its sign.

Detonator negativity at the synaptic knob

It is thinkable that a potential gradient similar to the spike-like potential gradient at the margin of the block is created at the synaptic knobs when impulses arrive there. It is true that if conduction through the knobs should take place in the same manner as in ordinary nerve fibers, the creation of a significant unidirectional potential gradient in the knobs, *i.e.*, in elements of microscopic size could hardly be assumed; but if the nerve impulse, when entering the fine branches of division of the presynaptic fibers and the synaptic knobs, should undergo a progressive modification comparable to the decrement observed at the margin of the block, then a steep potential gradient would appear at the knobs. This gradient would approximately have the duration of the spike process in the parent fiber and would vanish without reversing its polarity.

The spike-like potential gradient at the synaptic knob—henceforth to be called detonator negativity—would be responsible for the detonator action of the nerve impulse. Its duration would be that of the synaptic delay,—its maximum reached in a fraction of a millisecond,—and it would decay very rapidly so that effective summation of detonator action at neighboring knobs would be possible only when produced at very short intervals of time. In brief, a potential gradient of this kind would not only be a strong stimulus because it would force a dense current through the soma of the

neuron,[*] but it would also be a stimulus having all the known properties of the detonator action.

Response of the motoneurons to detonator negativity. It is to be expected that when the detonator negativity develops at the synaptic knob, a decremental wave of depolarization spreads over the neuron in a manner comparable to the spread of the spike-like negativity through and beyond the block that is shown by records 7 to 10 in Fig. 21. The depolarization must be greatest at the margin of the active knob and rapidly decrease with increasing distance. Except for the slight changes taking place during its propagation over the soma, the temporal course of the depolarization of the neuron should closely follow that of the detonator negativity at the synaptic knob and therefore, if the depolarization of the neuron has not been strong enough to initiate an all-or-nothing disturbance, it will disappear as fast as the detonator negativity itself.

Passive electrotonic transmission and local response in nerve An important question that must now be considered, but cannot yet be answered, is whether the transmission across the block of the spike-like negativity is attributable to "passive" electrotonic propagation or, in addition, involves an "active" response of the blocked segment of the nerve below the block Hodgkin (1937a, b) attributed the potentials recorded below the block to electrotonic transmission from the blocked impulse This interpretation was based upon the fact that subliminal electrical shocks were found to propagate across the block in a manner similar to that of the potential of the blocked impulse. The question, however, must be reopened because recent work (Hodgkin, 1938, Arvanitaki, 1938, and earlier papers) has led to the conclusion that in crustacean nerves the all-or-nothing disturbance is preceded by a local potential, that is considered to be an "active" response of the nerve fiber A "local" response at the cathode has been described for frog nerve by Katz (1937), although the evidence does not seem to be conclusive (Blair, 1938b) The conditions in multifibered nerves of vertebrates, however, are so much more complex than in crab nerves, that failure to demonstrate a local cathodal response may not be a conclusive proof that a local response does not develop before the all-or-nothing disturbance arises, but, on the other hand, it would be inappropriate to base theoretical arguments on the existence of a local potential in vertebrate nerve until some significant evidence of its existence has been found

One type of evidence accessible to present-day techniques would be a demonstration of differences in the propagation of the electrotonus away from the cathode and away from the anode It seems, however, that no apparent difference can be demonstrated [†] Fig 22 illustrates a pertinent experiment in which the propagation of electrotonus was measured in terms of the changes of excitability of the nerve Curves 1, 2, 3 and 4 measure the changes of threshold at the testing cathode (t) after delivery of a subliminal conditioning shock through electrode c For curves 1 and 2 the distance between the cathodes was two mm and for curves 3 and 4, seven mm For curves 1 and 3 the conditioning shock was 91 per cent of the threshold and for curves 2 and 3, only 30 per cent Despite the fact that according to Katz' (1937) observations, the stronger shock should have produced a significant local response, the transmission of the cathodal effect was similar in both

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[*] Since nerve fibers contain acetylcholine, there is no objection to the assumption that acetylcholine ions, as well as other ions, are involved in carrying the current, (cf Barron and Matthews, 1938, and Eccles, 1936, p 371). This movement of acetylcholine ions, however, would not result in a "release" of acetylcholine unless pathological conditions were created

[†] For a discussion of the problem of the spread of electrotonus cf Bogue and Rosenberg (1934) and for a similar problem that appears in the study of the conduction of the nerve impulse cf. Cremer, 1929, and Cole and Curtiss, 1939a, b.

cases, and it was also similar to the transmission of the anodal effect in the case of curves 5 and 6, although anodal shocks are not supposed to create "local potentials."

It seems, therefore, advisable to leave this important question open, especially because, in addition to those mentioned by Katz (1937), there still are other differences between the cathodal and anodal curves (cf. 1938e) that perhaps could not be explained without the assumption of a specific response at the cathode.

Residual negativity

The processes at the block do not end after the rapid decay of the spike-like potential, because this is followed by an enduring negativity that mimics the negative after-potential of a true impulse (Fig. 18, 1, 2; Fig. 19, 1a, 2a). This residual negativity is greatly increased when the block is conditioned by a train of impulses. Record 6 in Fig. 23 shows that while the train is being stopped at the block, each impulse adds a certain amount to the residual negativity left by its predecessor, with the result that at the end of the train there still remains at the margin of the block a significant gradient with the same polarity as the gradient created by the spike-like process. Records 7 to 10 in Fig. 24 demonstrate that this gradient is also transmitted through the block to points below. On the other hand, an examination of records 1 to 6 in Fig. 23 proves that the gradient at the margin of the block is also transmitted in the opposite direction (towards point 1). Since no records were taken from points between 4 and 7 (Fig. 24) it is not known at which point the maximal negativity would have been recorded; obviously however, it must

FIG. 22. Curves illustrating the transmission along the nerve of the effects of subliminal cathodal and anodal shocks. Green frog sciatic (Expt. 31-VII-37). The diagrams indicate the position of the conditioning (c) and testing (t) electrodes. In ordinates, height of the submaximal testing response conditioned by the subliminal conditioning shock at the intervals indicated in msec. in abscissae; unconditioned height indicated by the 100 per cent horizontal line.

1, 2, 3, 4. Cathodal curves. For 1 and 2, cathodes at two mm. distance; for 3 and 4, at seven mm. For 1 and 3, conditioning shock 91 per cent of the threshold; for 2 and 4, 30 per cent of the threshold.

5, 6. Anodal curves. Conditioning shock 91 per cent of threshold. For 5, distance between conditioning anode and testing cathode, two mm., for 6, seven mm.

have been maximal somewhere between points *4* and *6*. Therefore, it can be stated that in the whole nerve, immediately after the train of impulses, there was negativity in relation to point *11*, *i.e.*, to unchanged nerve, and that there was an increasing gradient of negativity from point *10* to points *6–4*, and from there on towards point *1* a decreasing gradient.

Similarity of potential at blocked segment with that at motoneurons after conduction of an impulse. A detailed consideration of the increasing gradient of residual negativity from points *10* to *6* (Fig. 21) will show that the block imitates a condition found in the motoneurons. As already mentioned, Wedensky demonstrated that when impulses enter a partial block they cause a rise in threshold in the block at the same time lowering the threshold below the block. Obviously, the demonstration of the rise of threshold at the upper margin of a complete block cannot be made with Wedensky's technique, but it can be obtained in oscillographic records. It will be noted in Fig. 19 that when the impulses are recorded above the block, the true spike potentials of all the impulses have the same height (Fig. 19, *3*); but when the spikes are recorded at or below the block, the spike-like potentials rapidly diminish in size (Fig. 19, *4*). This proves that the threshold at the margin of the block was raised by each successive impulse, so that the true conduction of each impulse ceased at a greater distance from the center of the block than the true conduction of its predecessor in the train, with the result that since the distance for decremental conduction was increased progressively, the size of the recorded spike-like potential decreased progressively. In records obtained at high sweep speed it was observed that the decrease in size was accompanied by a change in the shape of the potential
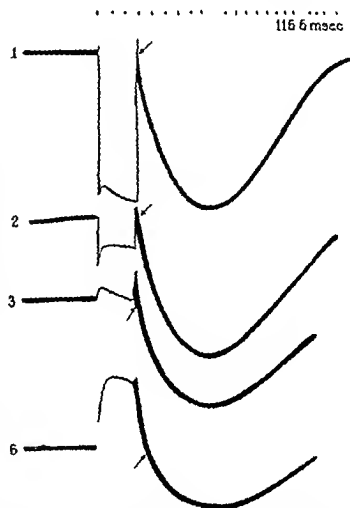
FIG 23 Cocaine block. Same experimetn as in Fig 20 and 21. Potentials recorded with the ground electrode successively at points 1, 2, 3 and 6 of the nerve, and the grid electrode at point 7 (Fig. 21). Stimulation by a train of shocks at the rate shown in Fig 20 Amplification five times higher than in Fig 21, and equal to the amplification in Fig 20 The shock strength in Figs. 23 and 24, however, was three times higher than in Fig 20. The tracings do not reproduce the spike-like potentials during the train, but indicate the lowest level reached by the spikes and therefore allow following the development of residual negativity. The arrows indicate the change of residual negativity into positivity.

and an apparent delay in the transmission that were like those observed in records *7* to *10* in Fig 21. In other words, the rise in threshold at the upper margin of the block produced the result that would be expected from displacing the recording electrode away from the block.

It is therefore clear that a partial block, after having been strengthened by transmission of one or several impulses, shows a distribution of potential (and also of thresholds) comparable to those observed during the phase of "standing" relative negativity of the motoneurons mentioned in previous paragraphs. (See Fig. 14 and 15.) In the block the greatest negativity is found at the upper margin which undergoes a rise of threshold, while the points below the block, which are electropositive in relation to the upper margin, undergo a lowering of threshold. Similarly, the soma of the motoneuron, after conduction of an impulse, becomes electronegative in relation to the axon and acquires a high threshold to synaptic stimulation (cf. Fig. 6, 2) while the axon, despite its being relatively electropositive, has an excitability to induction shocks that is either very near normal or even supernormal (cf. Fig. 6, *1*). Obviously, there is no contradiction between this theoretical analogy and the fact that when tested with induction shocks, the excitability of the soma parallels that of the axon (Lorente de Nó and Graham, 1938). During the phase of "standing" electropositivity of the axon there must exist potential gradients in the soma and, therefore, it may

FIG. 24. Cocaine block. Same experiment as in Fig. 20, 21, 23. Same stimulus and amplification as in Fig. 23. The grid electrode was maintained at point *11* of the nerve (cf. Fig. 21), the ground electrode was placed at the points indicated on the left of the records. The thin lines through the records indicate when the negativity changed into positivity at the different points of the nerve, and when the positivity reached its maximum. Note that the maximum of positivity appeared later, the greater was the distance below the center of the block. Further details in text.

be assumed that the current created by an induction shock may become effective at a zone of the soma comparable to points *7* to *10* (Fig. 21) of the nerve that is being taken as a model, while the synaptic stimuli may become effective at zones of the soma comparable to points *4* to *7* of the model.

Transmission of residual negativity. The transmission of the residual negativity through and below the block can hardly have been a passive elec-

trotonic conduction. In comparing record *6* in Fig. 23 with records *7* to *10* in Fig. 24 (cf. Fig. 20, *1* and *2*), it will be noticed that the transmission of the residual negativity is much slower than that of the spike-like potential (Fig. 21), and that the progression of the wave, of residual negativity, is not maintained entirely by the gradient at the upper margin of the block, because when at point *7* no negativity is recorded, about half the maximal negativity is recorded at point *10* (Fig. 24). Furthermore, the residual negativity is transmitted with much less decrement than the spike-like process (cf. Fig. 20, *1* and *2*). Under conditions such as these the conclusion is unavoidable that transmission of the gradient of residual negativity was not "passively" electrotonic. It must have been accompanied by an "active" response of the points of the nerve through which it was transmitted. The nature of the response cannot be ascertained from its electrical sign, or, in other words, electrical records do not reveal the nature of the processes that accompany the transmission; but taking into account the long duration and slow transmission of the process it seems to assume that the cumulative negativity was primarily attributable to changes in the polarized membranes, comparable to those taking place during transmission of the spike and spike-like negativities, but also that the transmission of the residual negativity resulted in a significant displacement of those ions that maintain the polarization of resting nerve.

Residual negativity at synaptic knobs could cause second phase of summation. An enduring and cumulative gradient of residual negativity created at the synaptic knobs would explain the second phase of lowered threshold of ganglion cells stimulated by a subliminal volley of impulses (c.e.s. in Eccles' terminology). Since in the model under consideration the transmission of residual negativity across the block is accompanied by a certain response, in order to preserve consistency of thought, it must be assumed that the neuron produces a similar response when it is acted upon by residual negativities at the knobs. It should be expected that this response would spread over the neuron in a like manner as the residual negativity spread through points *7* to *10* of the blocked segment of the nerve (Fig. 24). Near the active knobs the effect of the detonator negativity would be many times greater than the effect of the residual negativity (Fig. 19), but the difference would decrease with the distance from the active knob (Fig. 20). For the case of stimulation by a rhythmic series of impulses, the effect of accumulated residual negativity at points distant from the active knob could finally become almost as great as the effect of the detonator negativity of the first impulse of the train (Fig. 20, *1*, *1a*). Therefore, the neuron would acquire a persistently lowered threshold and the possibility of a temporal summation of impulses arriving in succession through the same group of fibers would be created.

Also, if the train had a duration and frequency beyond physiological limits, the cumulative effects of residual negativities could result in a rhythmic discharge of ganglion cells (after-discharge, cf. Bronk, in this Symposium), because the conditions in the ganglion would correspond to those

postulated by Adrian (1932) for rhythmic discharges initiated by peripheral end organs and injured ends of nerve fibers, as well as to the conditions studied by Arvanitaki (1938 and earlier papers) in crustacean nerves. During the phase of residual negativity significant displacement of ions may be expected (cf. Barron and Matthews, 1938) and, therefore, it is not difficult to imagine that one of concomitant phenomena could be the liberation of acetylcholine and other constituents (cf. Lissák, 1939) of the presynaptic fibers and synaptic knobs. Such liberation of substances, however, would occur after the completion of synaptic transmission by detonator actions and would only lower the threshold of the neuron and facilitate the transmission by detonator negativities of impulses arriving later. Obviously the release of cell constituents would outlast synaptic transmission (delayed output of acetylcholine, 1938a; cf. Barsoum, Gaddum and Khayal, 1934).

Electrotonic recording of residual negativity from segment of nerve above block. An interesting phenomenon is revealed by records *6* to *1* in Fig. 23 and *4* to *1* in Fig. 24. The gradient of residual negativity propagates itself not only toward the block, but also backwards toward point *1*, so that it can be picked up by two electrodes on normal nerve, the electrode nearer the block becoming electronegative in relation to the distant electrode. A similar argument is the basis of the method recently suggested by Barron and Matthews (1938 and previous papers) for recording electrotonically through spinal roots potentials developed in the spinal cord during activity; but it follows from the study of the conditions prevailing in the model that this method is not suitable for recording detonator negativities. Close analysis of Fig. 21 would undoubtedly reveal that the front of the spike potential, or even the whole spike, underwent a modification when approaching the block; but these records would not reveal in any other manner the development of the detonator negativity at the margin of the block. Only the existence of residual negativity would show itself in the form of a potential clearly different from that of the preceding spike process. This fact explains why Barron and Matthews (1938) do not describe detonator negativities.* Moreover, electrotonic recording from the roots cannot yield uncomplicated records if the same root is used for recording and leading. This fact is shown by the records in Fig. 23 and 24.

It will be noted in these records that immediately after the end of the train of impulses all the points of the nerve, when pitted against point *11*, *i.e.*, against an unchanged† point of the nerve, appeared to be electronegative. Owing to the spread of the residual negativity accumulated at the

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* Even when the electrotonic recording is made with a root adjacent to the root that has conducted the afferent impulses, according to the model, potentials attributable to detonator activities would easily pass unnoticed because detonator negativities are transmitted electrotonically with a great decrement, while the decrement of residual negativities is much smaller.

† "Unchanged" should actually read "as unchanged as it can be." Point *11* was 29 mm. below the center of the block and about 20 mm. above the cut end of the nerve. The demarcation current through it, therefore, was very small at the time of the observation, but it cannot be said that no change at that point had taken place at an earlier time.

margin of the block, the maximal potential was recorded at point 4, but the distribution of potential along the nerve changed progressively About 200 msec after the end of the train positivity was recorded from all points of the nerve and soon afterward, point 1 appeared as that having greatest positivity If this point had been pitted against point 4 a gradient of negativity would have been recorded because point 4 remained electronegative in relation to point 1 for the whole duration of the potential changes, that took place after the end of the train of impulses, but the recorded gradient between 4 and 1 would not have been a true sign of the changes at the margin of the block Immediately after the train of impulses, the difference of potential between 4 and 1 was attributable chiefly to electrotonic transmission towards normal nerve of the residual negativity that had accumulated at the margin of the block (Fig 23, 6), but later, when this residual negativity changed into residual positivity (cf the arrow on record 6, Fig 23 or the first thin line crossing through records 4 to 10 in Fig 24), the difference of potential between 4 to 1 must have been caused, to a significant degree, by the process underlying the true positive after-potential of the segment of the nerve (points 3 to 1) above the block (cf later)

Residual positivity

The positivity of the nerve above and below the block, when completely developed, had a distribution that was very different from that of the preceding negativity Immediately after the end of the train of impulses there was an increasing gradient of negativity from point 10 to point 6–4 and a decreasing gradient from point 6–4 to point 1, but the positivity distributed itself in a different manner because there was only one gradient of progressively increasing positivity from point 10 to point 1 Examination of the positive troughs of records 1 to 10 in Fig 24 reveals two important facts (i) The gradient was not linear, so that at each point of the nerve the amount of positivity developed was in a special ratio to the size of the previous spike or spike like potentials that was peculiar to each point of the nerve, this ratio being greater below than above the block (ii) The temporal course of the recorded positivity was different at each point of the nerve Another important fact is that, while the amount of residual negativity was not an exact indicator of the threshold at the various points of the nerve, pertinent tests showed that the positivity, above as well as below the block, was the sign of a raised threshold

Impossibility of successful analysis of residual positivity The model under consideration on the basis only of the observations thus far described, cannot be used for a direct attack on the problem A further analysis of the positivity is in fact, prevented by initial assumptions The creation of the residual negativity undoubtedly is attributable to certain changes of the polarized membranes of the nerve at the uper margin of the block, but it has been assumed that the transmission of the residual negativity, among other possible processes was accompanied by a significant displacement of ions Concentration gradients are by themselves a source of electromotive force, and as soon as they are produced they must establish a current through the input stage of the recording apparatus This unknown current will algebraically summate with the currents that owe their existence to altera

tions in the electromotive forces of the polarized membranes of the nerve. Therefore, the recorded potential difference, as it has a complex origin, may not be considered a true sign of any single process in the nerve. Thus, the much abused model, in reaching the limit of its present usefulness, leads to the discussion of an important problem, *i.e.*, that of interpreting potentials recorded with electrodes in contact with a tissue which is the site of processes resulting in the creation of electromotive forces.

Theoretical basis of interpretation of electrical records obtained from nervous tissue

As already indicated, this problem was treated with mathematical rigor by Helmholtz (1853 with review of the early literature) who established several principles that in the course of time have become elementary laws of electrotechnique. If a body within which electromotive forces have developed is placed in contact with an isotropic conducting medium, there is established in the medium a field of current that may be attributed to certain fictitious electromotive surfaces located at the physical boundary of the body and the medium. Where the current leaves the body there appears an electropositive surface, and where the current enters the body, an electronegative surface. Using a more recent form of speech, it may be said, "fictitious sources and fictitious sinks of current." The external field, *i.e.*, the field in the medium, is uniquely determined by the shape and electric properties of the fictitious sources and sinks of electricity (electromotive surfaces). Conversely, the external field, except for an irrelevant constant, uniquely determines the potential at the electromotive surfaces. In consequence, the external field of current is identical with the electrostatic field *in vacuo* between single electric layers with an electric density corresponding to the density of current at the electromotive surfaces. If the body is removed, but the electromotive surfaces are maintained by applying to the boundary of the medium suitable differences of potential that are created in any thinkable manner, the field in the medium will remain unchanged.

Therefore, analysis of the external field alone cannot reveal the nature or position of the true sources of electricity within the body where the electromotive forces are developed,* and an infinite number of true sources of electricity could be assumed that would produce the same external field, *i.e.*, the field measured in the medium. At the time of Helmholtz's writing (1853), knowledge of the structure of muscle and nerve was so incomplete that Helmholtz could make no assumption concerning the true sources of electricity. Still his treatment of the problem on the basis of the principles of mathematical physics led him to the then most remarkable statement that the demarcation currents that had been described for intact muscle could not be compared with the true demarcation current between normal and injured parts of muscle.

The diagrams of Hermann and Cremer. Twenty six years later when ad-

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* A lucid presentation of a similar problem is made with elementary mathematical means by Planck (1933, Chapter I and especially Chapter II). Cf. also, MacMillan (1930, with literature), and Kellog (1929, with literature).

vances of histological knowledge justified it, Hermann (1879) presented an illuminating diagram that was reproduced by Cremer (1909) and is reproduced here in Fig 25, III Hermann indicated that there were four think-
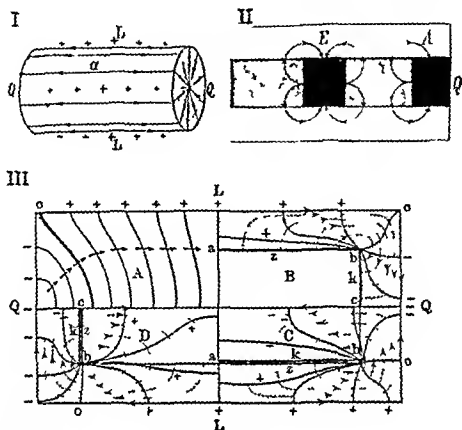
FIG 25 Diagrams of Herman (1879, Fig 47, 55, 48) indicating the bioelectric currents observed in muscle and nerve (I and II) and their possible origin (III)

I In muscle or nerve the longitudinal (uninjured) surface (L) is electropositive in relation to the (injured) cross sections (Q) Therefore currents are established in the directions indicated by the arrows

II The currents observed in nerve during activity E and after injury A Q, injured cross section The injured and the active segment become electronegative in relation to normal or inactive segments because currents are observed in the medium surrounding the fibers, in the direction indicated by the arrows

III While diagrams I and II represent observed facts, diagram III represents four "thinkable assumptions" about the electromotive surfaces responsible for the demarcation current A, a series of transversal electromotive surfaces of the indicated polarity, each reaching the surface of the element, B, an electromotive cylinder positive on the longitudinal section and negative on the cross section, C, double layer, positive outside and negative inside, distributed over the longitudinal section of the cylinder D, a double layer negative outside and positive inside, located at the (injured) cross section of the cylinder Hermann indicated that hypotheses A and B are ruled out by the fact that all the points of uninjured muscle are isopotential, between the two remaining assumptions, however, a selection could not be made by studying the external field of current Hermann favored assumption D (alteration theory of Hermann), but later studies have shown that assumption C is the correct one (membrane theory of Bernstein)

able kinds of true sources of electricity, and although he favored the source illustrated in diagram III, D (Fig 25), later research led to the admission of diagram III C, which embodies the main concept of the membrane theory Since at that time the existence theorems of the potential theory, which are usually known under the terms of Dirichlet's and Neumann's problems, had been demonstrated, it became possible, after assuming true sources of

current of known position and constitution, from measurements made in the external field, to reach conclusions concerning changes at the source of current, *i.e.*, the polarized membrane of the nerve fibers.

Thus, Cremer (1899) basing his argument on the mathematical calculations of Weber (cf. Hermann, 1884 and Weber, 1884) assumed that the field of current within a nerve surrounded by a dielectric was comparable to that in a linear conductor, and he was, therefore, able to show how from the density of current outside the nerve fiber, the density of current through the membrane and the change of electromotive force (polarization potential) at the membrane could be calculated by simple mathematical differentiations.

Factors that may complicate the analysis. In the study of the model of synaptic transmission in the phase of positivity a situation has been reached that is more complicated than the simple one considered by Hermann and Cremer. The existence theorems of the potential theory show that unique selection of a potential function among the innumerable functions which may satisfy conditions at a particular zone of the field demands knowledge of the values of the function or of its first derivative, not at any arbitrary surface through the field, but *at the boundaries of the field*. Given this boundary condition, the problem admits of one and only one solution; but if that condition is not given, an infinite number of solutions may be found, all of them having in common the values of the function at the fictitious electromotive surfaces of Helmholtz, *i.e.* at the surfaces of contact of the recording electrodes with the tissue.

Consequently, so long as it is assumed that the currents in the medium surrounding the active neuron are attributable exclusively to changes in the electromotive force in different parts of the membrane of the neuron, the records may safely be interpreted in the form that has been suggested while discussing Fig. 14 and 17 of this report. But if it be admitted that in the tissues other sources of electromotive force may develop, then the records do not admit of a unique interpretation. For phenomena of short duration, such as have been considered in this paper, it seems reasonable to assume that the main sources of potential are alterations of the electromotive force of the polarized membrane of somas and axons of nerve cells; but for long-lasting phenomena, which may involve chemical and concentration changes, the situation is different and a most careful analysis must be made before reaching a conclusion. The problem, however, does not seem as hopeless as it was in Helmholtz's time because, after all, the number of kinds of sources of electromotive force that may appear in nervous tissue is limited, and it may be hoped that careful mapping of the electric field in the brain tissue, in and around the active zone, will ultimately yield sufficient information to differentiate the various true electrical sources and investigate the changes they have undergone. In the case of the model of the synapse that has been subjected to study in this report, the available records measure only potential differences at the boundary of nerve and dielectric, but do not give any information about the internal field of the nerve trunk. Since the assumption has been made that during the transmission of the gradient of residual negativity, significant displacement of ions takes place, a knowledge of the field within the nerve trunk is necessary in order to ascertain how far the positivity is attributable to changes in the polarization of the membrane and how far to other electromotive sources.

Definition of true after-potentials

One more remark must be made before closing the analysis of the synaptic model, namely, that the analysis, besides other defects, has the great one of not having considered true "after-potentials." There was, however, a good reason for the omission. In the model, owing to the existence of the block, great potential gradients developed, and these seriously interfered with the study of after-potentials. Potentials that simulated after-potentials were observed, but they were not true after-potentials.

In agreement with the thesis developed by Gasser (1937 *a, b*) on the basis

of a considerable body of evidence, true after-potentials may be characterized as follows: After-potentials develop in nerve fibers following the conduction of spike processes, and their slow time course prevents their causing measurable potential gradients in normal nerve, because the entire length of nerve available for experimentation during the after-potential stays "in phase." Thus, after-potentials can be measured only by pitting points of the nerve that have been active against a killed end of the nerve separated from the live nerve by a sharp line of demarcation.

According to this definition the processes underlying the after-potentials are measured by their electric signs. But while the spike potential, besides being the sign of a process, is a stimulating agent, the after-potentials are purely signs of a "process of unknown nature, which affects the condition of the plasma membrane" (Gasser, 1937a, p. 167). Therefore true after-potentials, since they have been defined purely as signs of processes that among other things determine the course of recovery after activity, should not be directly homologized with potentials that act as agents. This statement perhaps needs clarification by an example. A nerve acquires supernormal excitability after conduction because its membrane during restoration undergoes a certain process, the sign of which is the negative after-potential; but it is not supernormal, because the negative after-potential is stimulating it subliminally. On the other hand, the segment of nerve below the cocaine block acquires a lowered threshold when the residual negativity gradient at the upper margin of the block acts upon it as a stimulus. This potential when recorded looks like a negative after-potential, but in a strict sense it is not purely the sign of changes that may be happening in the membranes of the nerve below the block and must be in part a propagated electric wave.

For these reasons the writer has carefully avoided the use of the term "after-potential" when describing the recorded differences of potential which, it is true, mimic after-potentials, but hardly can be considered as pure signs of the processes taking place when true after-potentials are recorded. Obviously, as the number of elementary processes that can take place in the polarized membranes of nerve fibers is limited, future research undoubtedly will establish relationships between the processes underlying the cumulative residual negativity and the following positivity, and the processes that take place in nerve after conduction to produce the negative and positive after-potentials. Perhaps it will become possible, when knowledge is sufficiently advanced, to use the same terminology for all processes that have similar electric signs, but until then it seems more advisable to use different terms for concepts as different as the negative after-potential, which is defined as a sign, and cumulative negativity, which has been considered in part a stimulating agent.

IV. Concluding Remarks

It is hardly necessary to emphasize that the preceding analysis of the cocaine block has consisted of a study of experimental facts, insofar as the

processes in the treated nerve were concerned, but that it became a theoretical argument whenever comparisons were made with processes happening at the motoneuron and its synapses. Nevertheless, it may be considered a useful analysis, because it has led to the examination of significant questions and has offered a statement of the electrical theory of synaptic transmission in a form that is suitable for detailed discussion and is capable of suggesting new experiments. The electrical theory, as presented in the light of the model, bridges a number of the differences in the views of recent investigators, which although in remarkable agreement in the interpretation of some phenomena, are in disagreement in the interpretations of other points. For example, the assumption of the production of two successive gradients of negativity of the synaptic knobs, if it were generally admitted, would unify the views of Adrian, Barron, Bronk, Bremer, Eccles, Gasser, Lorente de Nó, Matthews, and Sherrington. Detonator negativities with a temporal course similar to that of the spike process of the nerve impulse would cause a strong brief excitation; internuncial bombardment would insure restimulation by detonator actions and temporal summation. Residual negativities would also enter into the production of temporal summation. Moreover, residual negativities and, eventually, residual positivities, not only would be effective in producing changes in threshold, but would also result in environmental changes of the type demonstrated by Dusser de Barenne, McCulloch and Nims (1937). After the masterful analysis of Dusser de Barenne and McCulloch (1939) it is unnecessary to insist upon the harmonious blending of all the factors that take place during activity of the nervous system.

The model has perhaps done even more; it has emphatically shown that —as Gasser has indicated in the Introduction to this Symposium, and Erlanger and Bronk have pointed out in their contributions—the investigation in peripheral nerve of the properties of nervous tissue is a direct and profitable method of study of the elementary processes that take place in the nervous system. Knowledge of the properties of nerve may not result in the immediate solution of the problems offered by the central nervous system, but it supplies challenging analogies and working hypotheses.

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PROBLEMS OF SYNAPTIC FUNCTION

ALEXANDER FORBES

Department of Physiology, Harvard Medical School, Boston, Mass.

THE GREATEST achievements in science have been those which brought unity out of diversity. Observation reveals complexity and diversity everywhere. The great milestones are the discoveries of common trends or laws running through the complex pattern, or simple units of which it is built. Thus, while the biochemist has been giving us increasing molecular weights for the proteins, running now into millions, the atomic physicist has been reducing all substances to 3 or 4 units—proton, electron, neutron, etc. The animal body obviously possesses vast complexity, and no other system in it is so complex in structure or behavior as the nervous system. Twenty-five years ago, Keith Lucas (1917), after uncovering much of the nature of the functional response in nerve-fiber, faced the formidable array of functions which had been ascribed to nerve-centers with the question:

"Are we to suppose that the central nervous system uses some process different from that which is the basis of conduction in peripheral nerves, or is it more probable that the apparent differences rest only on our ignorance of the elementary facts of the conduction process? If we had a fuller knowledge of conduction as it occurs in peripheral nerve, should we not see Inhibition, Summation, and After-discharge as the natural and inevitable consequences of that one conduction process working under conditions of varying complexity?"

His answer to his own question was the suggestion

"that we should inquire first with all care whether the elementary phenomena of conduction, as they are to be seen in the simple motor nerve and muscle, can give a satisfactory basis for the understanding of central phenomena; if they cannot, and in that case only, we shall be forced to postulate some new process peculiar to the central nervous system."

This suggestion has found an echo more than once in physiological thinking since it was uttered—most recently in a schema offered by Gasser (1937 a, b).

Histology forces us to accept complexity of structure. There are many different kinds of synapse. Each afferent neuron divides into many end-branches. Histologists have recently estimated as many as 1300 end-bulbs on a single anterior horn cell. There is evidence that in general more than a single impulse in a single afferent axon is required to evoke a reflex response. Granting this, obviously even if each terminal branch brings to the synapse an impulse of all-or-none character, there is room in the above number of end-bulbs leading to the final common path to provide for a vast amount of gradation in the central excitation effect.

A recent monograph (Fulton, 1938) has said, "The simplest reaction of the nervous system is the segmental spinal reflex involving two elements, an afferent neuron and a motor unit." There is a temptation to simplify the picture thus for the portrayal of the sequence of events. But it is an oversimplification, which may be misleading since the most salient feature

of a reflex center seems to be branching and multiple interconnection, such that one afferent fiber makes many central connections and one motor neuron receives impulses from many converging paths. In a telephone system involving only two subscribers a central exchange would be superfluous; a single wire would suffice. To schematize the reflex arc as consisting of but two units is to miss the essential distributing property of the center.

Granting the high degree of structural complexity, which the pictures just shown by Dr. Lorente de Nó have emphasized anew, we face the question,—can we simplify the terms of the problem physiologically by finding any unity of function throughout the system? There is a highly significant unity in the conduction process common to nerve and skeletal muscle. In spite of the structural and functional differences between these tissues, all the essential properties in their mode of conducting the propagated disturbance are the same. This appears in the similarity of their excitation by electric currents. It is more strikingly revealed in the refractory phase and the all-or-none character of response—features which Adrian (1914) has shown to be causally connected, and which are both common to nerve and skeletal muscle. Finally, the similarity of the electric response or action potential is so close in the two tissues as to leave little doubt of a fundamental kinship in the underlying activities. Indeed, Lapicque (1926) has shown that the nature of electrical excitation, as attested by the curves relating strength and duration of the current required to excite, is essentially the same in a large variety of excitable tissues. Electric responses, in which the active region is negative with respect to inactive tissue, are also common to various excitable tissues of animals, including others besides nerve and striated muscle,—e.g., smooth muscle, gland and probably nerve-cells. This property, then, seems to be common to all animal tissues capable of functional response.

There was further admirable simplification when Erlanger and Blair (1931a, b) identified refractory phase in nerve with the electrotonic effect of current-flow—post-cathodal depression. A further step in the same direction is the evidence that, in nerve, facilitation is correlated with negative after-potential, and depression of excitability with positive after-potential (Erlanger and Gasser, 1937). Neatly correlated with that is the evidence of Hughes, McCouch, and Stewart (1937) that in the spinal cord inhibition is coincident with positive after-potential.

In the cervical sympathetic ganglion Eccles (1935a) reported a corresponding association of facilitation with negative after-potential, and inhibition with positive after-potential. But Rosenblueth and Simeone (1938a) in a thorough investigation were unable to substantiate these correlations. Their evidence pointed to the existence of other factors.

Sherrington (1925) emphasized the indications that in the center there is some process which differs from the nerve impulse in having no refractory phase and which instead is capable of accumulation by summation and thus of graded intensity. This hypothetical process he designated "central excitatory state" (c.e.s.), remaining noncommittal as to its nature. It might be

a substance whose concentration could be varied, or it might be the degree of depolarization of a membrane, polarized in its resting state. A substance is more easily visualized, and in the elaboration of Sherrington's idea it was designated by Fulton (1926) a "chemical theory." But "c.e.s." is still referred to in the noncommittal sense (Eccles, 1937a).

Chemical mediation of the effects of nerve impulses was first established in the case of vagus inhibition of the heart by the observations of Loewi (1921). Similar chemical mediation seems to be well established in the case of the activation of smooth muscle by the nerve impulse. In the case of skeletal muscle, the question is more controversial, but there is an impressive accumulation of evidence pointing to chemical mediation at the neuromyal junction.

In the sympathetic ganglia, especially the cervical, a great mass of evidence has been adduced to show the liberation of acetylcholine (ACh) in sufficient quantities to excite the ganglion cells (Kibjakow, 1933; Feldberg and Gaddum, 1933, 1934; Feldberg and Vartiainen, 1934), and the inference has been drawn that this substance is the synaptic transmitter (cf. Dale, 1934, 1935; Rosenblueth and Simeone, 1938a, b). The evidence in support of chemical mediation in the synapses of the central nervous system is not so clear, but if it is finally established that intercellular transmission is chemically mediated in such widely different systems as the neuromuscular junctions of smooth and striated muscle and the synapses of ganglia, the operation of a wholly different mechanism in the histologically similar synapses of the central gray matter would be a most surprising anomaly.

In spite of the strong evidence and arguments for chemical mediation, its claim to recognition as the synaptic transmitter has been seriously challenged. Much of the controversy has centered around cholinesterase, which exists in the tissues and has the property of destroying ACh by hydrolysis. Eccles (1937a, b) conceded that ACh increases the excitability of ganglion cells, but contended that cholinesterase could not destroy the ACh quickly enough to account for the rapid decay of the "synaptic transmitter" indicated by his observations. He reinforced this argument (1937a) by the failure of eserine (which protects ACh from cholinesterase) to prolong the action of the transmitter in his experiments. Rosenblueth and Simeone (1938b) found that with larger doses of eserine the decline of the transmitter (or mediator) is measurably retarded, and thus they removed one objection to the chemical theory. That the rapid decay of the transmitter may not be a serious obstacle will appear in view of other considerations to be mentioned presently.

The central excitatory state has been likened to the local excitatory process which Lucas (1917) postulated as the essential preliminary to setting up a propagated disturbance in nerve or muscle (Creed et al., 1932, p. 45). The resemblance rests chiefly on the fact that in each case there is evidence of gradation of the excitatory tendency and, by virtue of this gradation, of the cumulative effect of summation. In each case it appears that when the

excitatory state, or process, reaches a certain requisite intensity, it causes the discharge of an impulse. Certain evidence has been taken to signify that the central excitatory state (what Eccles now calls the "detonator action") disappears when it has reached threshold value and set up a discharge (Creed *et al.*, 1932, p. 44), or at least that "its subsequent course is submerged by the consequent refractory period" (Eccles, 1937a, p. 21). The implication is that it can never become supraliminal. Objection has been raised to the chemical theory on the ground that acetylcholine in large quantities can cause a continued repetitive discharge of motor neurons, which seems to be evidence of its persistence in supraliminal quantity and thus to render impossible its identification with the rapidly decaying synaptic transmitter. But it is quite possible that small quantities of ACh, sharply localized, can very quickly fall below threshold concentration, while a larger and more widespread production of it would make possible a persistent, supraliminal concentration. In this connection it has previously been suggested (Forbes, 1934) that a chemical mediator might be elaborated in a reservoir in which its quantity could vary according to the number of excitatory impulses producing it, yet at the point where it initiates the outgoing nerve impulses, it might never reach a supraliminal concentration, because in some way it would expend itself in the act of initiating a discharge. The reservoir might be the synaptic end-bulbs or the dendrites or the perikaryon. The point of initiating the outgoing nerve impulse might be the cell-membrane or the axon hillock.

When it comes to localizing events whose existence can only be inferred great caution is indicated. The argument of Eccles and Hoff (1932) that antidromic impulses pass backward over the cells has been summarized by Gasser as follows: "In their experiments, rhythmically discharging neurones have their rhythms altered by a volley back-fired into the cord; and an alteration in rhythm such as this would hardly be possible if the impulse did not get back to the point at which excitation of the neurone takes place" (Erlanger and Gasser, 1937, p. 189). It by no means follows from this evidence that the antidromic impulse traverses the entire neuron to its dendrites. Collision with the disturbance at various possible points within the cell might well cause interference. All we can safely conclude is that the antidromic impulse reaches the point at which the discharge is initiated, be it synapse, dendrite or axon hillock.

Concerning the disappearance of acetylcholine, even if its only means of dissipation is destruction by cholinesterase, the recent observations of Feng and Ting (1938) and the independent observations of Marnay and Nachmansohn (1937) have shown that in skeletal muscle cholinesterase is far more concentrated in the vicinity of the neuromuscular junction than it is in the rest of the muscle or than has been previously supposed. Feng and Ting conclude that its concentration is adequate for the rapid destruction of acetylcholine required by the chemical theory of transmission. These observations deal only with the neuromuscular junction, but there seems to be no reason why similar high concentration should not exist at the synapse

between neurons, where so many other features of intercellular transmission run a parallel course to that which has been found in the neuromyal junction.

In support of the electrical theory of synaptic transmission, Erlanger has just shown us that, in a nerve-fiber in which one or two internodal segments amounting to 1 or 2 mm. in length are blocked by anodal polarization, the action potential may restimulate the fiber beyond the inactive segment. In a further report on these observations Blair and Erlanger contend that, if the action potential can thus restimulate the axon across an inactive stretch of 1 to 2 mm., it is justifiable to conclude that it can excite the tissue beyond a synapse, "and that it will unless the synapse includes a device for preventing current spread" (1939, p. 105). In answer to this contention, it should be emphasized that the structural and presumably the electrical conditions are quite different in an unbroken but inactive axon from those at the synapse. Here, at the termination of the neuron, histology seems to reveal a transverse membrane, which may well act as a short-circuit to the action potential which is responsible for the effect observed by Blair and Erlanger. If the membrane theory of nerve conduction holds good, there is every reason to expect such a short-circuiting effect at the termination of the axon. Indeed, this anatomical consideration is perhaps one of the strongest reasons for seeking a different mechanism, such as chemical mediation, as an essential step in the excitation of the next neuron.

Lorente de Nó (see Forbes, 1936, discussion) has shown that a subliminal induction shock applied directly to a motor neuron and a subliminal afferent volley impinging on the same motor neuron can sum to produce excitation. He argues from this that the two exciting agents must be alike in kind and that therefore synaptic transmission is due to electrical excitation by the action potential. Yet, why should not a chemical substance which has the power of depolarizing the cell-membrane sum with an electric current which also depolarizes the same membrane? The evidence of community of effect is suggestive of a common cause, but not conclusive.

Rosenblueth and Morison (1937) in an investigation of the neuromyal junction of skeletal muscle showed that the effect of curare, eserine, and fatigue, and the phenomena of Wedensky inhibition, all present difficulties in the way of explaining transmission on the electrical theory, but are readily explained on the basis of mediation by acetylcholine, without resort to assumptions which have not been supported by experimental evidence. Cannon and Rosenblueth (1937) found essentially similar results in their observations on the cervical sympathetic ganglion, which strongly reinforce the chemical theory as applied to the synapse between neurons.

So goes the controversy. Dale in discussing it remarked that it was unreasonable to suppose that nature would provide for the liberation in the ganglion of acetylcholine, the most powerful known stimulant of ganglion cells, for the sole purpose of fooling physiologists. To this Monnier replied that it was likewise unreasonable to suppose action potentials would be delivered at the synapses with voltages apparently adequate for exciting the ganglion cells merely to fool physiologists. The consideration of the probable

short-circuiting effect of the termination of the neuron, mentioned above, appears to weaken the electrical argument and to strengthen the case for the chemical theory.

A review of this controversy between the electrical and the chemical theories of synaptic transmission recalls the experience of physicists in the study of light. When they perform an experiment designed to show that light is a wave-action, the experiment yields an affirmative answer and shows what it was intended to. When they perform an experiment designed to show the corpuscular nature of light, it also yields an affirmative answer. These results appear to present a direct contradiction, yet, for some reason which is quite beyond my powers of comprehension, the physicists seem perfectly happy about it and maintain that there is no real contradiction. I wonder if the electrical and chemical theories of synaptic conduction may also prove not to be mutually contradictory after all. Bronk (1939) has furnished evidence which leads him to a pluralistic view of synaptic function. Conceivably, we are dealing with electro-chemical events which have both electrical and chemical aspects, revealed according to the nature of the experiment. Every chemical effect is bound to involve a potential change and electrical forces in any system of chemical substances in solution must tend to produce motion of charged ions. There is evidence that potassium is liberated from a nerve when it conducts an impulse. Lissák (1939) has recently furnished evidence of the liberation of acetylcholine in active nerve-trunks. Brown and Feldberg (1936) showed that potassium chloride may stimulate ganglion cells and may also liberate acetylcholine. These observations suggest that we are dealing with a chain of electro-chemical events both in the axon and at the synapse and that the differences between them may possibly be differences in degree, as, for example, differences in the quantity of chemical substance liberated or in the distance it may migrate in different parts of the nervous structure. It has been suggested (Rosenblueth, personal communication) that the synaptic transmitter ("detonator action," according to Eccles) is acetylcholine, and that the slower and more prolonged effect which Eccles now designates "c.e.s." may represent a secondary change in the equilibrium of potassium ions in the system.

Much of the discussion has been based on the membrane theory of nerve conduction. It has already been pointed out (Forbes, 1936) that no anatomical structure in nerve has been identified with the supposed membrane, and that the observations of Cohn (1935) on the dipole moments of long protein molecules may perhaps give a clue to a more adequate theory of nerve function. Disturbances in the equilibrium of such molecules might conceivably produce effects which simulate the depolarization of a membrane. The place which acetylcholine might occupy in such a system seems to offer an interesting field for future research.

Summing up this question of the relation between the chemical and electrical events in the synapse, we might look for a sequence of events which, to use Lloyd Morgan's phrase (1901), presents a "dualism of aspect, distinguishable in thought but indissoluble in existence." But we must

not think so loosely as to overlook the fact that the electrical potential which excites a tissue is the same thing whether produced by a dynamo or by a galvanic cell, and this is quite distinct from acetylcholine,—a substance which can be put in a bottle, whatever its electrical properties

In conclusion, I may cite the observations reported last year by Renshaw, Forbes, and Drury (1938) in which micro-electrodes inserted into the pyramidal cell layer of the cat's hippocampus revealed two wholly distinct types of electric response. Smooth-contoured slow waves were derived from all parts of the hippocampus, whether the exploring electrode was in the axon layer, the cell layer, or the dendrite layer. When the micro-electrode was in close proximity to the cells, and only in that region, a wholly different type of discharge was also found. This consisted in monophasic spike potentials of approximately the same duration as axon potentials in the A fibers. Usually they appeared in groups of four or five, with progressively declining voltage and frequency, the whole group lasting only 15 or 20 msec. That these two types of discharge are wholly distinct phenomena is shown by the fact that no intermediate forms were found in any of our experiments; no correlation was seen between them. The time-relations of the quick responses suggest the discharge of impulses from the cell-body. The fact that the micro-electrode adjacent to the cell always became negative during these rapid spikes suggests localized depolarization or negativity over the surface of the cell-body, or a part of it, with reference to the more widely distributed portion of the cell, e g., the dendrite.

The chemical and anatomical complexity of the nervous system should warn us to beware of adopting too readily alluring schemata with any confidence that they really will embody the truth about nervous function. It will be necessary to keep an open mind about various theories for a long time to come. As yet, we have hardly any clue to the switching mechanism whereby such things as volition are achieved, that is, the way in which one path or another may be open to the streams of impulses under the varying conditions which occur in the life of animals.

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PHYSIOLOGICAL ANALYSIS OF THE GENERAL CORTEX IN REPTILES AND BIRDS*

F. BREMER, R. S. DOW†, AND G. MORUZZI‡

Laboratoire de Pathologie Générale, Université de Bruxelles, Belgium

I. INTRODUCTION

THE FUNCTIONAL significance of the so-called general cortex of reptiles and birds is not well understood. Physiological studies are few and the use of the oscillograph for study of the cortex of these submammalian forms has not been attempted. The extensive and frequently controversial literature concerning this part of the telencephalon will not be reviewed; a brief description of the principal anatomical facts is, however, essential for orientation. For a complete and critical treatment of the anatomical problems the reader is referred to the work of Kappers, Huber, and Crosby (1936), and for physiological literature to the recent reviews of ten Cate (1936, 1937). Previous work directly concerned with the present experiments of stimulation and oscillographic recording is discussed

ANATOMY

Reptiles The general cortex, or that part of the surface area not primarily olfactory in character, lies in the dorsal lateral surface of the cerebral hemisphere Its medial boundary is formed by the hippocampal region and its lateral boundary by the piriform lobe It is in intimate relation by means of a primordial neopallium with the hypopallial region which is probably homologous to certain parts of the corpus striatum of mammals Dart (1935) has subdivided this general cortex into a medial para hippocampal portion and a lateral para piriform area One of the most complete studies of the anatomy of the reptile telencephalon is that of Johnston (1915) on the turtle This rather primitive reptile is more easily homologized with the amphibian forms and primitive mammals than are certain other species which seem to have developed along avian lines Johnston was able to outline a general cortex extending over the dorsal and lateral surfaces of the hemisphere This area separates the hippocampal and piriform areas except at the rostral pole where these olfactory regions fuse He felt that the medial part of the cortex was principally sensory, while the lateral and anterior parts were mainly concerned with motor function, but recognized the probability of considerable overlapping between the two

Besides the interconnections between the general cortex and its contiguous areas, both cortical and hypopallian, this area has numerous afferent and efferent connections with the diencephalon and mesencephalon Various workers have described four tracts from the hippocampus and associated regions going by way of the median forebrain bundle to the hypothalamus and adjacent areas of the mesencephalon These are considered predominantly efferent in respect to the cortical areas and include within them connections homologous to the fornix and fornix lungus systems of mammals and the septo mesencephalic system in the bird There are numerous pallial commissures connecting the olfactory regions of the two hemispheres The question of the presence or absence of a true corpus

* The term general cortex or general pallium was introduced by Johnston in 1915 in his description of the turtle brain Kappers, Huber and Crosby (p 1338) state "The term general cortex . is applied to those cortical areas which are concerned primarily with impulses other than olfactory, although such general cortex will be associated by fiber bundles with the developing olfactory cortices medial and lateral to it "

† Fellow of the Belgian-American Foundation

‡ Fellow of the Rockefeller Foundation
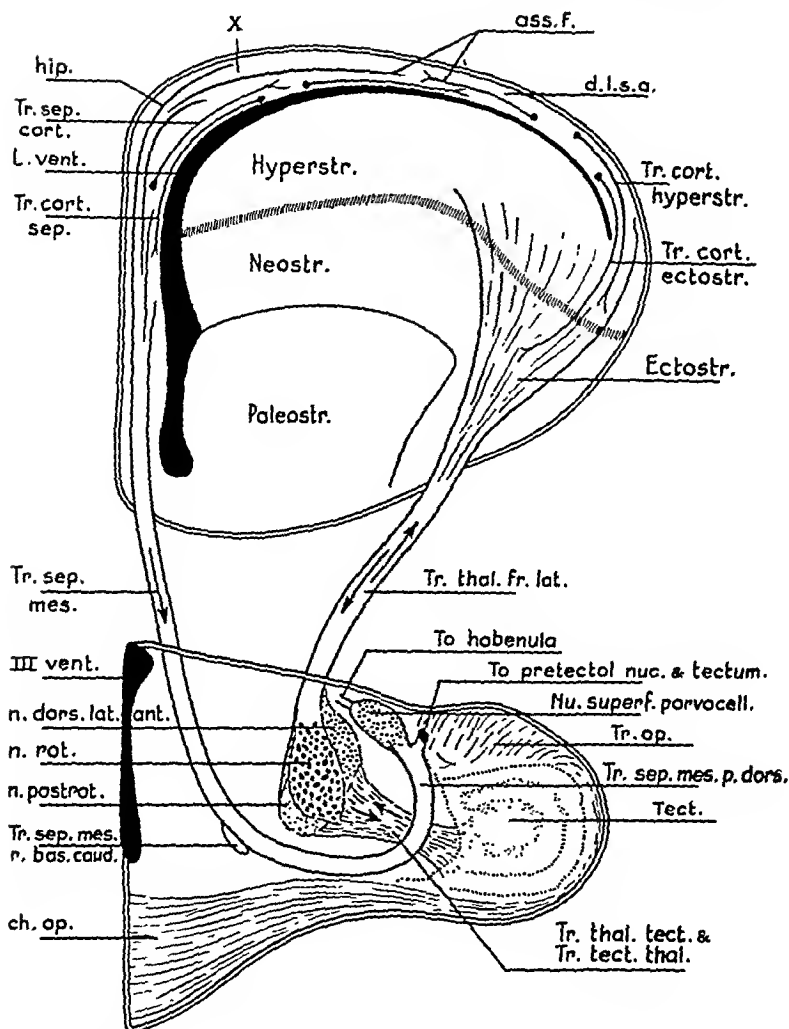
FIG. 1. Diagram to show the principal connections of the dorsolateral surface area (corticoid area) in the pigeon. (Modified from Kappers, Huber and Crosby, 1936.) ass. f., association fibers; ch. op., chiasma opticum; d.l.s.a., dorsolateral surface area; ectostr., ectostriatum; hip., hippocampus; hyperstr., hyperstriatum; l. vent., lateral ventricle; n. dors. lat. ant., nucleus dorsolateralis anterior; n. postrot., nucleus postrotundus; n. rot., nucleus rotundus; n. superf. parvocell., nucleus superficialis parvocellularis (nucleus of the tractus septo-mesencephalicus); neostr., neostriatum: paleostr., paleostriatum; tect., tectum; tr. cort. sep., tractus corticoseptalis; tr. cort. hyperstr., dorsolateral surface area component to the hyperstriatum; tr. cort. ectostr., dorsolateral surface area component to the ectostriatum; tr. op., tractus opticus; tr. sep. cort., tractus septocorticalis; tr. sep. mes., tractus (cortico-) septo-mesencephalicus; tr. sep. mes. p. dors., tractus (cortico-) septomes-encephalicus pars dorsalis; tr. sep. mes. r. bas. caud., tractus (cortico-) septo-mesencephali-cus ramus basalis caudalis; tr. thal. fr. lat., tractus thalamo-frontalis lateralis; tr. thal. tect. and tr. tect. thal., tractus thalamo-tectalis and tractus tecto-thalamicus. III vent., third ventricle; X, area of uncertain homology. (See Kappers, Huber and Crosby, 1936.) The connections between the dorsolateral surface area and the hyperstriatum accessorium are not shown. The olfactory connections of the septal region are not shown, nor is the tractus septo-mesencephalicus ramus basalis frontalis indicated.

callosum in these forms is unsettled and must await the application of degeneration experiments Numerous connections between the cortex and the habenular region have been described within the stria medullaris The principal non olfactory afferent connection to the general cortex is probably carried by ascending tracts in the lateral forebrain bundle, which is well developed in reptiles Of particular importance are the fibers in the extreme lateral part of this complex bundle Shanklin (1930) has described a direct connection between the dorsolateral cortex and the nucleus rotundus, but this has not yet been found by other workers The paths carrying the impulses responsible for the motor movements produced by stimulation of the dorsal pallium in reptiles have not been demonstrated (Kappers Huber, and Crosby, 1936) *Association fibers within the general cortex area* are well developed The question of cortical lamination in reptiles is still one of controversy Its identification apparently depends both on the criteria considered essential for identifying such structural organization by different investigators and on the species examined Lamination in the mammalian sense is not present in the general cortex of the turtle

Birds The telencephalon of birds differs from that of the reptiles in the relative reduction of the olfactory connections in most species and in a great development and differentiation of the corpus striatum This has gone hand in hand with the increased differentiation of the dorsal thalamus and other somatic centers particularly the optic system The general cortex h

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area (Fig 1) Edinger, Wallenberg, and Holmes (1903) and others have labelled in addition the hyperstriatum accessorium and the dorsal hyperstriatum as cortex Following the definitions of the neopallium as given by the students of mammalian cytoarchitectonics, Rose (1914) maintained that there is no avian neopallium It is obvious that an opinion whether there is or is not a neopallium depends on one's definition of the term The importance of comparative physiological studies to supplement anatomical observations is clear The brain of the pigeon has been exhaustively studied by Edinger, Wallenberg, and Holmes (1903) in an admirable comparative anatomical work and by Huber and Crosby (1929)

The connections of the avian telencephalon have been determined by degeneration experiments, as well as by the silver and myelin staining methods The principal connections of the dorsolateral surface area are shown in Fig 1 The first tract to be described was the cortico septomesencephalic tract It has been studied by degeneration experiments (Boyce and Warrington, 1898, Edinger and Wallenberg, 1899, and Wallenberg 1906, and others) and the facts of its origin and termination are well established It is an efferent tract in respect to the ' cortex' and distributes to the hypothalamic regions and to the basal regions of the mesencephalon and even the medulla in certain species by its basal ramus The tectum and the habenula receive its dorsal ramus Though not morphologically resembling the pyramidal system of mammals, it may well serve a similar function in the avian forms The other connection with the lower centers is the lateral thalamofrontal tract This is probably both ascending and descending, although the tract has not been studied as systematically as the septo-mesencephalic The lateral thalamofrontal tract probably provides a connection to and from the well developed tectal region and the cortex' by way of the nucleus rotundus and other neighboring nuclei Its lateral location in respect to the ventricle and *its striatal course* resembles the *internal capsule system of* mammals Its functional significance, although probably important, is not well known, but it is thought that the ascending connections are of chief importance Short association neurons are found within the dorsolateral surface area and connections to the adjacent areas of hippocampal cortex and striatum are numerous and undoubtedly important functionally (Fig 1)

It is obvious that although the avian telencephalon bears a certain resemblance to the mammalian its homologies are less clear than are those of the more primitive reptilian telencephalon The "cortex" has apparently become vestigial to some extent, while the striatum has developed in complexity and differentiation, and parts of it probably serve as a vicarious cortex As we shall see, however, although anatomically less well developed, the corticoid layer of birds still bears a functional relationship to the homologous parts of the mammalian brains

Reptiles. Properly speaking, there is no physiological literature on the cerebral cortex of reptiles. The small size of the cortex and its intimate union with subcortical centers has rendered a selective study of the general cortex extremely difficult. This has forced us to consider the reported physiology of the entire forebrain and to attempt to separate that part which concerns the cortex proper. The experiments of *ablation* are inconclusive. The older studies of Fontana, Rolando, and Desmoulins were inconclusive (ten Cate, 1937). In the turtle, after ablation of the cerebral hemispheres, spontaneous movements were less frequent (Fano, 1884, and Bickel, 1901), but nevertheless possible (Sergi, 1904). Following such ablations in the adder, according to Schrader (1892), there were no longer movements corresponding to various emotional states such as fear, rage, etc. The responses to light stimulation were normal (Bickel, 1901).

Stimulation experiments have furnished more abundant results, the interpretation of which is difficult, because of the danger of spread of the current. Bickel (1901) never obtained any effect in the turtle with the use of weak electrical stimulation or with chemical stimulation. Only strong stimulation gave a response, and this was probably attributable to spread of current. Johnston (1916) on stimulation of the anterior part of the brain of the turtle obtained movements of the neck, eyes, jaw, extremities, and tail. Stimulation of the olfactory bulb and the striate body likewise induced these movements. The other parts of the forebrain were inexcitable. Analogous results were obtained in the lizard. The anterior part of the pallium has a particular histological structure and this might suggest that it was the excitable part of the forebrain. However, the fact that narcosis augments rather than suppresses the movements made him think that this action results from a spread of current. The observations of Koppányi and Pearcy (1925) confirm this point of view. These authors have not observed in the turtle movements from electrical stimulation, except when the electrodes were forced into the corpus striatum, and in this Tuge and Yazaki (1934) concur. They have confirmed in the turtle most of the phenomena described by Johnston (1916). Bagley and Richter (1924) and Bagley and Langworthy (1926) have described complex movements, never discrete, on stimulating a well localized part of the cerebral cortex of alligators. Narcosis diminishes the excitability of this zone, and, moreover, stimulation of both the striate body beneath and other cortical areas about the excitable area does not produce this effect. This difference in behavior between the alligator and the turtle may be explained by the fact that the brain of the latter is less well developed.

Birds. The same general remarks that have been made for reptiles apply here. Because of the relatively greater development of the striatum, ablation experiments, which have uniformly included these parts of the forebrain, have no value in the analysis of the function of the cortex in birds. The experiments of excitation are numerous, but opinion is divided concerning the excitability of the cortex. Ferrier (1876), confirmed by Stiner (1891) and Boyce and Warrington (1898), described an area in the superior parietal region of the brain, the stimulation of which provoked myosis of the contralateral eye and a rotation of the head to the opposite side. Gallerani and Lussana (1891) have also observed some movements of the head on chemical stimulation of the posterior part of the cerebral hemisphere. The most complete research is that of Kalischer (1900 a and b, 1901, and 1905), who studied carefully the electrical excitability of the cerebral cortex in various birds, particularly in the parrot. In this last species where the cerebral cortex is particularly well developed, Kalischer found evidence of true motor localization. Proceeding from a rostral point caudally, a focus was observed for tongue and jaw movements, a center for phonation, foci for movements of the foot and wings, and finally in the occipital region a zone was present which gave movements of the eyes.

Negative results are not lacking, however. Bickel (1898) has never been able to observe movements from weak stimulation, but only with the use of strong currents. He has considered them as attributable to spread of current to the striate body. Koppányi and Pearcy (1925) have likewise recently reported some negative results in the pigeon. Rogers (1922) working on the cerebral cortex of the pigeon, arrived at the same conclusion. He found only two movements of certain cortical origin, namely myosis of the contralateral eye and a depression of the feathers about the throat. All other movements were interpreted as being attributable to a spread of current to the corpus striatum. The divergence of results is striking, especially in so simple an experiment. The cause of the negative results is probably due to the use of the narcosis. Roger's experiments were performed under ether

anesthesia, and it is possible that this author has not obtained the responses of the most excitable centers, these having been depressed by the ether The experiments of Bickel and those of Koppanyi and Pearcy have all been done on unanesthetized animals In these experiments, there may be possibly another cause of error, i e, the pain provoked by the immediately preceding operation

The pigeon and the turtle have been used as experimental animals All observations of excitation and derivation of potentials have been made in the absence of all general anesthetics The animals were prepared under ether narcosis generally some hours before the beginning of the experiment An apparatus was constructed that could be affixed to the head of the animal and permitted the fixation of electrodes for stimulation or derivation but did not disturb the movements of the head In experiments in which both stimulation and derivation were done, the same electrodes were used, but the preparation was shunted from the amplifiers The animals were completely awake during the experiment, but were kept free from pain by the appropriate local use of 2 per cent cocaine

The stimulation was either monopolar or bipolar A faradic current with a frequency of about 40 to 50 per sec was used The derivation of potentials was always done with bipolar electrodes made of silver wires to which were attached fine cotton wicks soaked in Ringer's solution The electrical activity was registered by means of an amplifier of five stages coupled with small capacities (0 1 μF), and a Dubois oscillograph

II Experimental Studies

Stimulation

Turtle Faradic stimulation of the brain provokes movements of the neck, jaw, and feet The intensity of the stimulus is of the same order as that necessary to induce masticatory movements when applied to the masticatory area of the cerebral cortex of the normal rabbit However, these motor effects in the turtle are certainly not cortical in origin The use of cocaine applications indicates that they are probably due to a diffusion of current to subcortical centers We were unable to abolish the responses in question by a very prolonged cocainization (2 per cent applied for 5 min) It is known that 2 min are sufficient to abolish the responses from the masticatory cortex in the unanesthetized rabbit It will be pointed out later that only 5 sec of this cocainization are sufficient to abolish all motor responses from the cortex of pigeons

Pigeon The faradic stimulation of the cerebral cortex of the pigeon without general narcosis has given definite and conclusive results Under these conditions, the excitability is high and the responses are readily abolished with the most superficial application of cocaine The movements that were induced by weak stimulation of the dorsolateral surface area of the forebrain of the pigeon are the following (i) Rotation of the head toward the side opposite to that stimulated (ii) Conjugate movements of the eyes in the same direction (Forward movement of the homolateral eye and a backward movement of the contralateral eye) (iii) Myosis of the contralateral eye (iv) Depression of the contralateral lower eyelid

The first three movement were constantly present and usually simultaneous The rotation of the head resembled a "spontaneous" movement of the animal It might be carried to 180° This movement of rotation often included a vertical component either upward or downward The lowering

of the contralateral lower eyelid was not seen in every instance. Figure 2 shows the extent of the excitable area. We were unable to separate special centers within the excitable area for the different movements. The threshold of excitability was low. With bipolar stimulation (electrodes cotton wool wicks soaked in Ringer's solution) a distance of 25.5 cm. between the primary and the secondary coils of the inductorium usually gave a response. With the same apparatus, electrodes and frequency and strength of stimulation (2 V. in the primary and 50 shocks per sec.), the threshold of the masticator cortex in the waking rabbit is generally with the coils 24 cm. apart. The threshold is still lower, in the pigeon as in the rabbit, if a punctiform metallic monopolar electrode is used.

If, having determined the intensity of the stimulation that was exactly supraliminar, the frequency of the breaks in the primary circuit were reduced from 50 to 2 per sec., a response was still seen. In these conditions however, instead of the response appearing in 2 sec. as in the first instance, it did not appear until the stimulation had been prolonged for 30 to 40 sec. With the stimulation at this low frequency, the response although it was long delayed in appearance, otherwise did not differ from the movement produced by the more frequent stimulation. The movement of rotation of the head did not show any discontinuity corresponding to each separate induction shock.
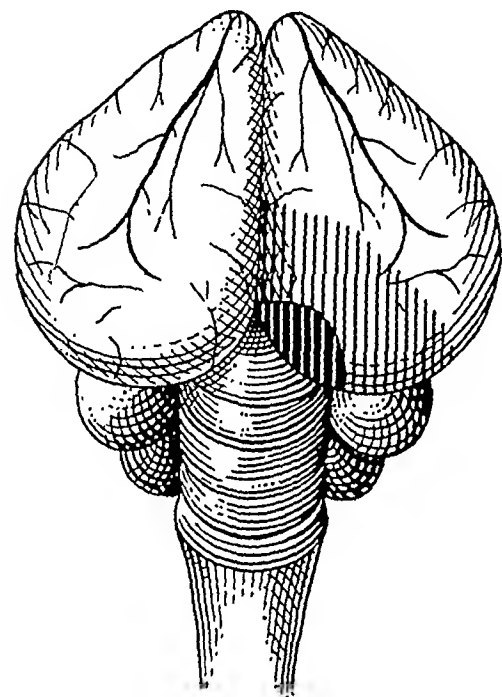
FIG. 2. The topography of the excitable zone of the cerebral hemispheres in the pigeon. The region with black stripes on a white background indicates the limits of the excitable area. That with white stripes on a black background is the region of maximal excitability.

The excitability of the cerebral cortex is sensitive to outside agents. General narcosis of even light ether or dial reduces it considerably. In addition, in the absence of general narcosis, if pain was not prevented by the appropriate use of a local anesthetic (cocaine 2 per cent), it was observed that the responses were much less regular. This was perhaps caused by a nociceptor inhibitory reflex. We believe that these factors are the principal causes of the divergence between the reported observations by different workers on this problem.

All the above mentioned responses were immediately abolished by an extremely superficial cocainization. If a piece of filter paper only 5 mm. square, moistened in a 2 per cent solution of cocaine, was placed on an

excitable point for only 5 sec. the response from this point was completely suppressed. This procedure is much more rapid and exact than when cocaine is applied to the laminated cortex of mammals. The adjacent points of the cortex about the area cocainized remained excitable, showing that the effect of the cocaine was restricted to the site of its application. Another proof of the cortical nature of this response has been furnished by chance. One of the pigeons of this series had an atrophy of the cortical tissue on one side in the area giving the responses (See protocol below.)

EXPERIMENT, PIGEON NO 4

After a preliminary ether anesthesia, the left hemisphere was exposed in the usual manner by trephine and rongeur On opening the dura it was obvious that the dorsolateral area of the occipital and parietal region was abnormal The corticoid layer was thinner than normal, wrinkled and translucent There was no sign of recent damage to the bone, meninges or skin over this area The opposite hemisphere was exposed, as was customary, and was normal in gross appearance. Inasmuch as the atrophic area coincided so closely with the area from which movements of the head were obtained, we proceeded with the following experiment

Technique Inductorium with 2 V in the primary and 50 break shocks per second Monopolar metallic electrode at the point of maximal excitability Duration of stimulation at each strength, 2 sec As a test of response a movement of the head toward the contralateral side For cocainization a piece of filter paper 5 mm sq, moistened with 2 per cent cocaine Responses were obtained from the normal side (right) with weak current while even prolonged stimulation with much stronger current on the atrophic (left) side failed to give any response Cocainization abolished the response on the right side

After the stimulation experiment, the superficial surface of both sides was hyperemic and slight lesions had unavoidably been made at one or two points on each side The brain was carefully removed from the skull and examined grossly. The medial part of the tissue outside the ventricle was identical to its fellow of the opposite side, but the lateral portion, the dorsolateral surface area proper, was less than one-half the normal thickness The frontal parts of the brain and the striatal areas, as well as the cerebellum and hindbrain, appeared to be normal and everywhere symmetrical The brain was imbedded in celloidin and sectioned at

FIG 3A Section through the dorsolateral surface area on the left side (Expt 4), the most superficial layer is a blood clot, the result of stimulation and trauma The two arrows mark the limits of the corticoid layer B A section through the opposite side in the same area Note the thickness of the corticoid layer as compared to 3A C Diagram of section from which photographs 2A and B were taken

fully removed from the skull and placed in 95 per cent alcohol After fixation it was cut transversely and examined grossly. The medial part of the tissue outside the ventricle was identical to its fellow of the opposite side, but the lateral portion, the dorsolateral surface area proper, was less than one-half the normal thickness The frontal parts of the brain and the striatal areas, as well as the cerebellum and hindbrain, appeared to be normal and everywhere symmetrical The brain was imbedded in celloidin and sectioned at

25 micra, every 12th section being mounted and stained by the Nissl technique for nerve cells.

Histological examination failed to reveal any abnormality of the frontal pole of the telencephalon, nor any of the striate areas, the extreme lateral or medial parts of the "cortex" or any lower center. However, from the most anterior point at which the dorso-lateral surface area is separated from the underlying hyperstriatum by the lateral ventricle, the intermediate portion of the cortex, corresponding to the position of the general cortex in reptiles, was obviously thinner than its corresponding layer of the opposite side. Figure 3A and B, shows sections through corresponding portions of the atrophic and normal sides respectively, taken near the rostral border of the defect, at the junction of the anterior two-thirds and posterior third of the hemisphere. Figure 3c is a diagram of the entire section, showing the site of the microphotographs 3a and b. More posteriorly, the atrophic area is even thinner and in places indistinguishable from the underlying caudal neostriatum at low magnification.

The motor responses described are thus cortical in origin. To ascertain whether there are correlations between the two excitable areas on the two sides, an attempt was made to reproduce the phenomena of "secondary facilitation." In pigeons, however, the simultaneous stimulation with currents just subliminal was without effect. There is, therefore, probably no mutual reinforcement between the two cortices, and the absence of the phenomenon of secondary facilitation speaks against the existence of important intercortical correlations in these forms. Simultaneous stimulation with two physiologically equal but supraliminar stimuli resulted constantly in a curious effect which was probably the result of an interaction of the cortical excitation on the subcortical centers. In these circumstances, the animal turned its head neither to the right nor toward the left, but made movements of the head and neck toward the front, as if to peck at some object with its beak. All these movements, like those produced by unilateral stimulation, were similar in appearance to spontaneous movements of a normal animal.

Oscillographic studies

Turtle. The normal oscillogram in the unanesthetized turtle should be compared to a similar method of recording of the potentials derived from the exposed brain of an unanesthetized mammal. The rabbit has been the only animal previously studied (Ectors, 1936). The amplitude of the electrical potentials is much less in the turtle than in the rabbit. In general, we have been obliged to work with a sensitivity of 5 to 10 times that ordinarily employed in mammals. The aspect of the oscillogram is much more regular. One does not see, in contradistinction to the rabbit, the large waves comparable to the human electroencephalogram. In the turtle there is simply a succession of fairly regular electrical oscillations of a frequency analogous to the beta waves (about 40 to 50 per sec.) and of feeble amplitude (10 to 20μV., Fig. 4A).

The electrical activity thus recorded is not exclusively cortical. A small part of it does have its origin in the cells of the cortex proper, but the potentials of subcortical centers are recorded simultaneously. A superficial cocainization does not suppress or even reduce the activity considerably. After the application of 2 per cent cocaine for 5 to 15 min. there is still considerable spontaneous activity. It is only when concentrations of 5 to

10 per cent of cocaine are employed that one is able to abolish, reversibly to be sure, the electrical activity (Fig. 4E–F). It is known that cocainization for 5 to 10 min. with 2 per cent solutions is sufficient to abolish almost completely the spontaneous activity of the area striata in the cat (Claes, 1939).

The oscillogram of the turtle is thus an encephalogram, and not a corticogram, as with the use of this method of leading in mammals. The intimate spatial relations between the cortex and the subcortical centers are the essential cause of this difference. We have nevertheless studied the behavior

FIG. 4. The local action of strychnine and cocaine on the electroencephalogram of the unanesthetized turtle. 100 μV—100 mm. in the original. 2/3×.

A. Normal activity.
B and C. One minute after the end of a local application of strychnine sulfate 1/1000 (duration of application 2 min.). Note the violent discharges which were unaccompanied by motor manifestations.
D. Immediately after B and C. Bursts of increased electrical activity without convulsive characteristics. The discharges and bursts of activity alternate in the oscillogram.
E. After 2 min. of local application of 2 per cent cocaine. The basal electrical activity remains, although both manifestations of strychnine action have disappeared.
F. After 2 min. of local application of 5 per cent cocaine. Note the disappearance of all electrical activity. This effect is reversible; after some time the activity reappears.
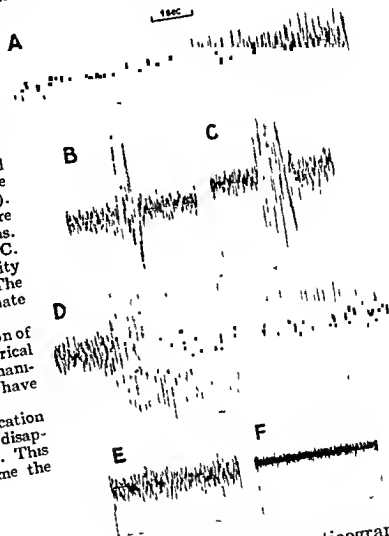
of the electroencephalogram of the turtle in relation to the corticogram or mammals in different experimental conditions. Strychnine in solution of 1/1000, applied to the cerebral cortex of mammals produces characteristically large, rapid, electrical potentials, the so-called "strychnine spikes" of Kornmüller (1935, 1937 see ref. 31), Gozzano (1935), Bremer (1935). This drug applied to the cortex of the turtle acts in an entirely analogous fashion. After these typical strychnine pulsations there was at times a considerable after-discharge lasting 3 or 4 sec., which gradually decreased in amplitude. It seems as if the neurons were not exhausted after the convulsive discharge, but continued to discharge more or less asynchronously. In the mammalian corticogram this phenomenon is not generally observed after convulsive discharges produced by strychnine or epileptic in character. In
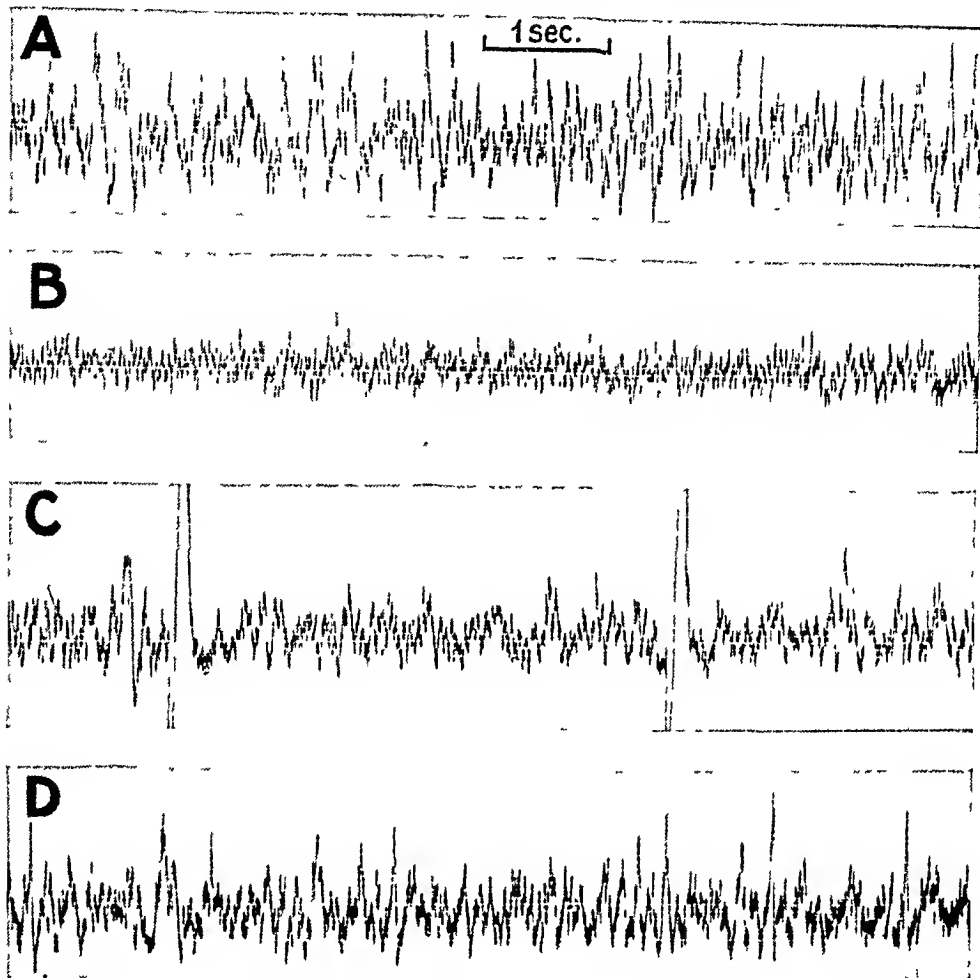
FIG. 5. The local action of strychnine and cocaine on the electroencephalogram of the pigeon. 100μV—15 mm. in the original. No reduction.

A. Normal activity.

B. 30 sec. after the local application of strychnine 1/1000 for 1 minute. Note the diminution of amplitude of the waves and an increase in their frequency.

C. 3 minutes later. Note the "strychnine spikes."

D. Between C and D 2 per cent cocaine was applied locally for 10 sec. Note the disappearance of strychnine discharges, but conservation of the spontaneous activity.

consequence, the question arises whether in the turtle this activity, which we have called "after-discharge" may not in reality be a response of subcortical centers that have been put into activity by the strychnine-induced convulsive discharges of the cortical neurons.

This hypothesis seems to be confirmed by the experimental observations illustrated in Fig. 4. In this experiment the application of 1/1000 strychnine solution to the cortex of the turtle's brain for 1 min. caused the appearance of the typical giant, abrupt discharges, which are altogether analogous to those in mammals (Fig. 5B–C). Some seconds later, one witnessed the ap-

pearance of bursts of activity of great intensity lasting about 5 sec., but without convulsive characteristics. This increase in activity is an augmentation pure and simple of the frequency and amplitude of the normal waves (Fig. 5D). These bursts of activity and convulsive strychnine discharges ("strychnine spikes") alternate in the oscillogram. The local application of 2 per cent cocaine for 2 min. caused the complete disappearance of the strychnine discharges. After an interval the other bursts of activity gradually disappeared also. But the basal activity persisted despite the cocainization (Fig. 4E). Only a 5 per cent solution applied for 2 min. finally resulted in the complete disappearance of activity (Fig. 4F).

Continuing our research, the effects of faradic stimulation on the electroencephalogram of the turtle were examined, a study that has previously been made on mammals. According to Dusser de Barenne and McCulloch (1937), a faradic stimulation is followed by a phase of depression in the spontaneous activity at the point of stimulation. This depression is difficult to explain, because it is simultaneous with the period of primary facilitation which, at least in part, is a cortical phenomenon. In reality the observations of Dow (1938) on the cerebellum of the cat, and of Moruzzi (1938) on the masticator cortex of the rabbit, show that, allowing for an interval for stabilization of the amplifiers, an augmentation of electrical activity in the area that has been stimulated takes place immediately after a faradic stimulation. It is true that at times there is a depression of activity under certain conditions, and in the case of the cerebellum, depression always follows the phase of augmentation (Dow, 1938). Furthermore, an apparent depression of activity may be characterized by a diminution of the amplitude, with an increase in the frequency of the waves, a condition probably produced by the desynchronization of the neurons (Moruzzi, 1938).

In the turtle, faradization of the cortex was followed by a definite afterdischarge, which, no matter how strong the stimulus, was never epileptiform in character. In this regard it resembled more closely the after-discharge from stimulation of the cerebellum than of the cerebral cortex in mammals. It is needless to emphasize that this after-discharge may not be, and probably is not, exclusively cortical. We have attempted, although unsuccessfully, to obtain from the cortex of the turtle the effects of "on" and "off" seen in the area striata of mammals with optic stimulation.

Pigeon. The general aspect of the oscillogram derived from the cerebral cortex of the pigeon (Fig. 5) is not fundamentally different from that of the rabbit. The irregular alteration of the large alpha waves with the small frequent waves of the beta type is seen in the bird, but the amplitude of the pulsations is much smaller. As in the case of the turtle, and probably for the same causes, in the pigeon there is simultaneous appearance of electrical activity of the subcortical centers. In consequence, we have an electroencephalogram, and not an electrocorticogram as in mammals. The spontaneous activity was not abolished by superficial cocainization, even though we have shown the extreme sensitivity of the excitability of the cortex to cocaine to be greater than in the case of mammals. It is improbable

and counter to all that we know on this point, that a profound depression of excitability such as this should be without effect on the spontaneous activity. One must then again turn to the conclusion reached in the case of the turtle, that under these circumstances of derivation, subcortical as well as cortical potentials are being recorded.

The action of strychnine in the pigeon is analogous to that in mammals. It is characterized by abrupt, ample, electrical potentials, the so-called "strychnine spikes." Immediately after the local application of strychnine we observed, in accord with what has been described for mammals (Bremer, 1936), an intensification of the preexisting spontaneous activity, without modification of its form. At times this intensification was masked by a diminution of amplitude that results from the desynchronization of the neurons and was then manifest only by a great augmentation of frequency (Fig. 5B). To this phase was quickly added that of the strychnine pulsations—abrupt, ample, and typically spike-like (Fig. 5C). As in the turtle, these strychnine pulsations in all probability are the expression of the activity of the cortex, or at least of the superficial layers of the cerebral hemispheres. The application of 2 per cent cocaine for only

FIG. 6. Epileptic activity of the cerebral cortex produced by faradic stimulation at the site of derivation in the unanesthetized pigeon. 100 μV—15 mm. in the original. ×5/6.
  A. Normal activity.
  B. Immediately after intense faradic stimulation for 5 sec. Note the violent epileptic discharges.
  C. Immediately after B. The epileptic activity has disappeared. The waves are slightly less in amplitude and slightly more frequent than in A.

10 sec. caused their immediate disappearance (Fig. 5D). This light cocainization had no important effect on the basic spontaneous activity.

The action of faradization of the brain on the electrical activity varied with the intensity of the stimulus. With weak stimulation it was difficult to produce an after-discharge of sufficient duration to be seen after stabilization of the amplifiers at the end of the stimulus. If a more intense stimulation was given, there was seen an after-discharge characterized by large waves of an epileptiform nature (Fig. 6). This after-discharge was of brief duration and at times was followed by a depression of the spontaneous activity. We have never been able to observe the phenomenon, so frequent in the rabbit (Moruzzi, 1938a), of the transformation of this after-discharge into a prolonged Jacksonian epileptic attack. Knowing the great development of the optic system in the bird and the important connections of this system to the telencephalon, we have studied with particular interest the

cortical reactions to illumination of the contralateral eye. We were able to obtain the "on" effect regularly, but consistently unable to obtain a clear "off" effect. The effects did not disappear after superficial cocainization of a duration (15 sec.) which suffices completely to abolish the motor responses. Only a prolonged cocainization was sufficient to abolish these effects. It has been shown by Claes (1939) that the effects "on" and "off" in the area striata of the cat are less susceptible to the superficial action of cocaine than is the spontaneous activity. Moreover, spontaneous activity in general is more sensitive to depressing agents than is provoked activity (Bonnet and Bremer, 1937; Moruzzi, 1938 b and c). Perhaps these facts may be used to explain the observations made on the pigeon. However, because of the presence of subcortical centers immediately subjacent, we must not forget another hypothesis, namely, that the effects observed are not cortical in nature, but entirely subcortical. We have no experimental data which might serve to decide this question one way or the other.

SUMMARY

1. The cerebral cortex of the turtle, *Emys europea*, appears to be electrically inexcitable in the usual sense of the term, *i.e.*, there is an absence of visible motor reaction attributable to the excitation of cortical neurons.

2. The electrical activity of low voltage which it is possible to record from the cerebral cortex of the unanesthetized turtle is essentially subcortical (striatal) in origin. However, the ability to produce "strychnine spikes," which are rapidly abolished by superficial cocainization, suggests the existence of a cortical component.

3. The cerebral cortex of the pigeon is electrically excitable by a weak current. The reactions to a unilateral stimulation consist in a conjugate deviation of the head and eyes toward the opposite side, accompanied by a myosis and opening of the palpebral fissure. This response is the expression of the excitation of neurons of the cortical layers, because: (i) the response is abolished almost instantaneously by the superficial cocainization of the excited region; and (ii) an animal which presented a congenital aplasia of the cortex on one side, as verified histologically, did not react to the application of strong stimulation applied to this side, while the opposite cortex which appeared to be normal gave the usual response to weak stimulation. These conclusions concerning the reality of motor reaction dependent upon the cerebral cortex of birds confirm and justify by new evidence the studies made by older workers, particularly those of Ferrier and Kalischer. The negative results reported more recently are possibly explained by the use of light anesthesia or by the inhibition resulting from failure to apply cocaine to the borders of the wound in the unanesthetized animal.

3. The simultaneous excitation of two symmetrical points on the right and the left cortex of the pigeon with currents of equal intensity results in rhythmic movements of the head in the vertical plane, suggestive of pecking movements in the intact animal.

4. The spontaneous electrical activity derived from the cerebral cortex of the unanesthetized pigeon resembles closely that of the rabbit when awake. The partial resistance of this activity to superficial cocainization indicates that it includes a subcortical component. The superficial strychninization of the cortex causes the appearance of the large "strychnine spikes" which are abolished rapidly by superficial cocainization. A brief cortical faradization releases a short after-discharge of epileptiform type.

5. The cerebral cortex of the pigeon reacts to the illumination of the contralateral eye by a large initial wave (effect "on"). The cessation of the illumination does not provoke a distinct "off" effect.

6. The general conclusion derived from an analysis of the excitability and the electrical activity of the cerebral "cortex" of the turtle and the pigeon indicates that, in the pigeon at least, the superficial layers of neurons covering the striatum dorsally and posteriorly have physiological properties and a functional significance—the latter being essentially opto-kinetic—much like those of the neo-pallium of mammals.

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STUDIES IN THE PHYSIOLOGY OF THE EMBRYONIC NERVOUS SYSTEM:
IV. DEVELOPMENT OF ACETYLCHOLINE IN THE CHICK EMBRYO*

ZING YANG KUO

*From the Nutrition Laboratory of the Carnegie Institution of Washington
Boston, Massachusetts*

INTRODUCTION

THAT ACETYLCHOLINE (ACh) is liberated when cholinergic nerves are stimulated seems well established. Recent evidence suggests that ACh is also liberated at central synapses and at neuromuscular junctions of striated muscle, but it is still unsettled whether liberation of ACh by electrical stimulation is a coincidence—*i.e.*, merely a product of increased metabolic activities of nervous tissues possibly associated with pathological processes (Fleisch *et al.*, 1936; Lorente de Nó, 1938)—or whether the presence of such a chemical substance is actually concerned with transmission of impulses at synapses and at neuromuscular junctions. Study of the origin and development of ACh in embryos, and its correlation with anatomical development of the nervous system and of reflex activities may throw light on the question. The chick embryo appears to offer favorable material for this purpose, since information is already available concerning the development of its nervous system and since the development of the chick's reflex movements is known (Kuo, 1938).

MATERIALS AND METHODS

Chick embryos of 2 to 12 days were used. Embryos of a given age were collected and placed in a dish containing eserinized standard Ringer's solution. The embryos were quickly cut into small pieces and thoroughly ground. The ground mixture was immediately tested for ACh. In those experiments for determination of ACh, the concentration of eserine varied, and no attempt was made to dilute the mixture in proportion to weight of embryo. In identification experiments only mixtures from 3-, 4-, and 5-day embryos were tested. Tests were made on frogs' hearts (Straub method), frogs' rectus abdominis muscles (Chang and Gaddum, 1933), and on dorsal muscles of leeches (about 15 segments). Both frog's rectus and leech muscle were bathed in 2 cc. of eserinized Ringer's solution, the concentration of eserine being 1:300,000. However, in those experiments conducted to estimate the quantitative variations of ACh from day to day during embryonic development, the following procedure was rigidly followed: (i) Only frog's rectus abdominis was used. (ii) The concentration of eserine in the bathing fluid was 1:300,000. (iii) The concentration of eserine contained in the embryonic mixture was 1:50,000, since higher concentrations of eserine gave better ACh yields. (iv) The embryos were weighed before they were cut and ground. (v) The mixtures were diluted with Ringer's solution in proportion to the weights of the embryonic tissues, so that equal weights of embryonic tissues of different ages would tend to have equal volumes of mixture (Table 1). (vi) All tests were made immediately after the tissues were ground, since standing tended to increase the ACh in the mixture.

Results

The identification of ACh. With the exception of the paper of Youngstrom (1938) there has been no previous report in the literature of a systematic investigation of the developmental history of ACh in the embryo. Hence it seemed desirable to employ every existing physiological method for the certain identification of the ester. All six methods proposed by Chang and Gaddum (1933) were used, and all results were positive and con-
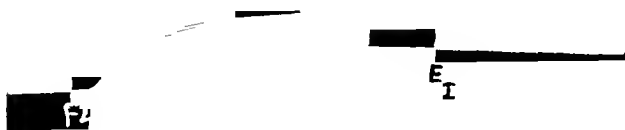
Fig. 1. Responses of frog's rectus to eserinized embryonic (chick) extract and to plain extract. F-4 illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, and E₁ the response to 1 cc. of plain embryonic extract of 4 days of incubation.

Fig. 2. Responses of frog's rectus to eserinized embryonic (chick) extract treated with weak acid and with weak alkali. F-5 illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, to which 0.1-normal HCl (about 4 drops per cc.) was added. I illustrates the response to 1 cc. of eserinized embryonic extract of 4 days of incubation, to which 0.1-normal NaOH (about 4 drops per cc.) was added. In both cases after HCl or NaOH was added the extract was boiled, cooled, neutralized, and tested with litmus paper before being assayed on the rectus.

sistent. Figures 1 and 2 give samples of results. It can be concluded that the substance assayed in the embryonic mixture was ACh.

Quantitative changes of ACh during embryonic development. Table 1 gives the quantitative estimates of daily variations of ACh during embryonic development. The tests were made with embryos of 2 to 12 days, inclusive. The table reveals that ACh can be detected in the embryonic tissues as early as 2½ days. It is highly probable that if sufficient embryos younger than 2½ days had been available, it would have been possible to detect ACh in the embryo of 2 days or even younger. There is a great increase in the amount of ACh per embryo from day to day during incubation. Such in-

crease is more apparent than real, however, for there is no significant and regular increase or decrease per unit of weight from the fourth to the twelfth day of incubation (Table 1, last column). The broken-line curve in Fig. 3

Table 1. *Quantitative changes of ACh during the first 12 days of incubation.*

Incubation in days	Total number of embryos	Total weight in gm.	Total diluted extract in cc.	Total amount of ACh in γ	Amount of ACh per embryo in γ	Amount of ACh per gram of total weight in γ
2	484	2.18	5	0.00	0.0000	0.00
2.5	468	6.16	15	2.04	0.0044	0.33
3	480	8.21	20	3.41	0.0071	0.42
4	120	7.92	20	6.18	0.0515	0.78
5	44	7.91	20	7.44	0.1691	0.94
6	18	8.28	20	6.32	0.3511	0.76
7	11	8.21	20	7.27	0.6609	0.89
8	10	12.18	30	9.50	0.9500	0.78
9	10	16.21	40	15.60	1.5600	0.96
10	10	23.81	60	21.11	2.1110	0.89
11	8	26.72	65	24.05	3.0063	0.90
12	6	27.81	70	22.47	3.7450	0.81

(page 492) shows more clearly that between the fourth and the twelfth day the production of ACh per gram of tissue fluctuates irregularly and that the quantity of daily variation during this period is relatively small.

Correlation with development of nervous system

A search of the literature dealing with the morphological development of the chick's nervous system revealed one fact clearly: *i.e., no synapse is found in any part of the nervous system up to the end of the third day of incubation.* Although fibrillogenesis in the central nervous system begins as early as the 38th to the 42nd hour of incubation (Cowdry, 1914; Tello, 1923; Windle and Austin, 1936), the fibers are short and few, even in the 3-day embryo, and there is no indication of the appearance of synapses. Comparably, primary sympathetic trunks do not appear until the end of the third day or the beginning of the fourth, and the *anlagen* of the secondary sympathetic trunks do not arise earlier than the sixth day (His, 1897; Abel, 1910; Kuntz, 1910). When the primary sympathetic trunks first appear, moreover, they are merely aggregates of cells.

ACh thus appears long before any synapses are formed and at a time when the bulk of the neural tube is still made up of epithelial cells and germinal cells not yet differentiated into primitive nerve cells, when neuroblasts are still relatively few, and when neuro-fibers are still short and few. Furthermore, as development proceeds, the length and number of neuro-fibers, synapses and neuromuscular junctions increase from day to day, whereas the relative amount of nervous tissue in proportion to other tissues decreases rapidly. In the early stages, brain size is enormous in proportion to other parts of the body, and the head is extremely heavy. But the ratio of head

weight to total body weight falls from 67 per cent on the 4th day to 30 per cent on the 12th, and to 18 per cent on the 20th (Kuo, 1932b). However, in spite of these progressive morphological changes, the increase of ACh per gram of tissue from the 4th to the 12th day of incubation is so irregular and uncertain that there is no apparent correlation between the development of ACh and the development of the nervous system (Fig. 3, broken-line curve).[1]

Correlation with development of reflexes

In the chick no movements, spontaneous or in response to reflex stimulation, can be observed before the 4th day of incubation, and reflex activities increase rapidly in kind, magnitude, and frequency per minute during the first half of incubation (Kuo, 1932a, 1938). Table 2, giving average fre-

Table 2. *Average frequency of spontaneous reflex movements per minute in the chick during embryonic development.*

Days of incubation	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Reflex movements	0.02	1.4	8.7	11.6	12.3	11.1	7.4	4.8	4.6	3.9	3.4	3.8	3.6	3.5	3.1	2.8	3.4	3.4

quency of spontaneous reflex movements per minute during incubation, is based on data accumulated by the writer during recent years. Comparison of this table with the last vertical column of Table 1 shows that there is no indication of a correlation between the development of ACh and the development of reflexes during the period studied; ACh appears long before reflex movements and then shows an irregular fluctuation from the 4th day on, whereas reflex movements increase rapidly and regularly from the 4th to the 8th day and then steadily decrease. This lack of correlation is illustrated more clearly by Fig. 3.

DISCUSSION

In view of such discrepancies between anatomical maturation and the concentration of ACh in relation to the appearance of reflexes, one is tempted to conclude that ACh, as it is found in a mixture of embryonic tissues, is not concerned with transmission of nerve impulse in the embryo. Such a conclusion does not preclude the possibility that the choline ester liberated at a particular moment of nervous excitation may act as a transmitter at synapses or at neuromuscular junctions. Such a possibility, however, remains to be proved. The mere presence of ACh or even an increase in

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[1] The results and interpretations of the investigation by Nachmansohn (Thomas and Nachmansohn, 1938; Nachmansohn, 1938, 1939) on the development of choline esterase in the chick embryo are of uncertain significance, since he appeared unfamiliar with the developmental history of reflex movements (Kuo, 1932a, 1938) and did not begin his investigation until the incubation period was nearly half over (9 days). Moreover, his data are incomplete during the second half of incubation, since he used only 9-, 12-, 16-, and 20-day embryos. The amount of tissue he used for his tests was probably too small to warrant conclusions.
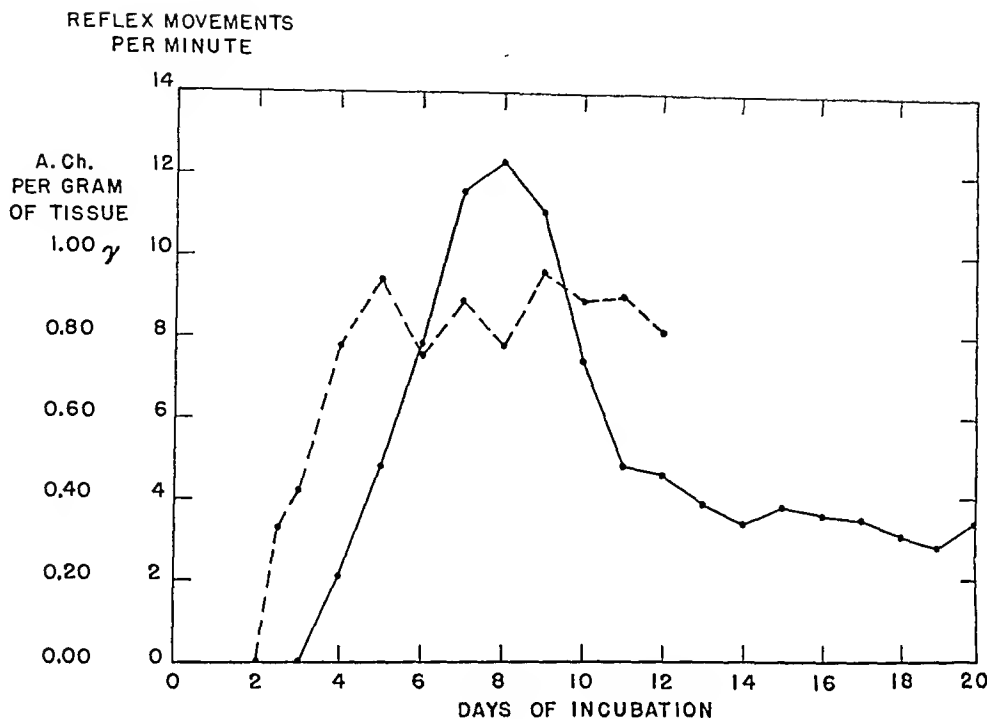
Fig. 3. Comparison of development of ACh with development of reflex activities in the chick embryo. The full-line curve shows the average spontaneous reflex movements per minute and the broken-line curve the daily variations in ACh per gm. of tissue during embryonic development. The curves are plotted from the data in Tables 1 and 2.

its output at nerve terminals or synapses at the time of excitation is no sure evidence that the ester is actually concerned in transmission. The fact that ACh exists in most animal tissues, including such nerveless structure as human placenta, and can be detected when hydrolysis of the substance is prevented, has not been given satisfactory explanation by the proponents of chemical transmission.

The present results create a more difficult drawback, which can not be lightly dismissed as irrelevant to the theory. They lend support to the demand for better evidence that accumulation of ACh is not merely a result of increased metabolic activity or injury or other pathological process. The claims made by MacIntosh (1938a, b) and by Bacq and Coppée (1938), that in degeneration experiments there is a time at which both the preganglionic fibers and ganglion cells appear to be still functionally intact whereas transmission across the synapses no longer occurs (which event coincides with the disappearance of ACh from the ganglion), are important in this connection, but they must be more thoroughly and systematically reinvestigated before they can be fully evaluated. The view that liberation of ACh is a general phenomenon associated with metabolic activities and is not in any way peculiar to nervous excitation also lacks decisive proof.

The results of Lorente de Nó (1938) are significant but not conclusive and have been contradicted by a more recent investigation (MacIntosh, 1938b) The evidence from the chick embryo here reported strengthens the metabolistic point of view but does not disprove the transmission theory

SUMMARY

1 Tissues of chick embryos were ground and assayed for acetylcholine (ACh)

2 ACh increases from $2\frac{1}{2}$ to 4 days of incubation and then shows random fluctuations until the 12th day

3 There was no indication of a positive correlation between the development of ACh in the chick and the development of reflexes and of the nervous system

4 The bearing of the results on the theory of chemical transmission is discussed

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EFFECTS OF ACOUSTIC STIMULI ON THE WAKING HUMAN BRAIN*

P. A. DAVIS, M.A.

From the Department of Physiology, Harvard Medical School
Boston, Massachusetts

INTRODUCTION

SPECIFIC immediate electrical responses of the cerebral cortex to peripheral stimulation have been described for the anesthetized and the unanesthetized animal (see References for principal citations). More general and persistent modifications of pre-existing activity, such as the initiation or the checking of fast frequencies are also familiar (Bartley and Heinbecker, 1938; Bremer, 1937a, b, c). In the human, the checking of the alpha rhythm by opening the eyes, and, less regularly, by a sudden sound, was described by Berger (1929) and widely confirmed by others (see References). But immediate positive responses in the human to sounds or other stimuli have received only scant attention, partly because they do not appear under all conditions and partly because observers have feared being misled by artefacts due to muscular movement and particularly movements of the eyes (cf. Rohracher, 1938; Travis, Knott, and Griffith, 1937; and Wessell and Carmichael, 1938).

The present observations, made in 1935–36, now take on a special interest because of their close relationship to the responses of the human brain during sleep which are described in another paper (Davis, Davis, Loomis, Harvey, and Hobart, 1939). They were obtained in 41 experiments on 38 adult men and women.

METHODS

A single-channel Grass amplifier with an "undulator" type of ink-writer (Garceau and Davis, 1935) was employed. Our silver-silver chloride electrodes were approximately 5 mm. in diameter. Sanborn "Redux" electrode paste was rubbed into the scalp, and collodion was used to hold the electrodes in place. Reference electrodes made like ear rings were applied to both ear-lobes, and connected in parallel. Standard placements on the head were the vertex and the occiput 2 cm. above the inion in the mid-line. A few experiments included, in addition, the mid-frontal region, 6 cm. on either side of this region, and the temporal areas.

A Clough-Brengle beat-frequency oscillator and a loud-speaker were the source of the sound. A signal recorded automatically on the tape the onset and duration of the stimulus. Both loud and faint tones in the range from 250 to 2000 cycles were employed. Standardization of experimental conditions included a consideration of the subject's physical comfort and his physiological state of alertness. This requires that the subject be free from fatigue or drowsiness before the experiment starts. Careful explanation of the purpose and each step of the procedure were given so that the subject would understand and be free from apprehension.

Procedure. While the subject lay comfortably on a bed in the softly lighted room, he was asked to open and close his eyes several times. The purpose of this was to have an electroencephalographic record of the subject's eye-movements, his responses to light and to the sound of the experimenter's voice when asked to open and close his eyes. The

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sequence of tones was 250, 500, 1000, and 2000 cycles, each being given twice, first as a faint, then as a loud tone The duration of each tone was a few seconds and the interval between tones a longer period The sequence was then reversed Finally, the subject was told that the sound stimuli were going to be changed and put in irregularly without any preconceived arrangement This was done

RESULTS

Electrical responses to sound stimuli cannot always be detected When observed they are of the same character regardless of the area in which they appear The response was seen more clearly from the occiput than from the temporal or frontal areas, but most prominently from the vertex (Fig 1)
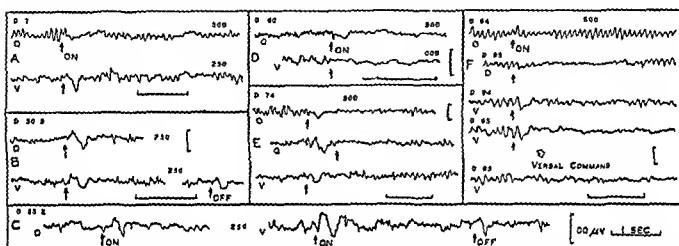
FIG 1 On effects and modifications of spontaneous rhythms in response to sounds The frequency employed is indicated in each case No measurements of loudness were made O = monopolar occipital record, V = monopolar record from vertex Reference electrodes on ear lobes connected in parallel Paired tracings do not represent simultaneous records Calibrations are for 1 sec and 100 μV throughout

A—checking of alpha rhythm and on effect in an alpha subject
B—on and off effects in a non alpha subject Note also checking of beta waves in vertex record
C—same as B in another subject
D—checking of fast (beta) frequencies in another non alpha subject
E—typical on effects "Anticipatory" reaction in second line
F—checking of alpha rhythm and on effects in a pair of identical twins, D 94 and D 95, also effect of a verbal command

The response was an "on-effect" composed of a diphasic, and sometimes triphasic, wave During the first phase the active electrode becomes electrically more negative The total duration of the on-effect was approximately 0 3 sec or less The voltage, measured from the peak of the negative phase to the trough of the positive phase, ranged from 100 μV to deflections which were just distinguishable from the background of physiological activity The latencies could not be precisely measured but were of the order of 30 to 40 msec (see Fig 1)

An "off-effect" sometimes occurred upon cessation of the tone (see Fig 1B and C), and was similar in character to the on effect (Fig 1B and C) It did not occur as often as the on-effect, but sometimes appeared upon cessation of a stimulus which produced no visible on-effect It was rarely as prominent as the on-effect

On-effects appear in both the alpha and the non-alpha type of individuals (Fig. 1, A and F, B and D). In non-alpha subjects, the on-effect can be seen more easily due to the lack of alpha rhythm. In alpha subjects, the only response to sound often appears to be a momentary checking of the alpha rhythm (Fig. 1, A 1st line, and E 1st line). When the speed of the tape is doubled, the checking of the alpha rhythm can be studied in greater detail. The wave-length and shape of the alpha rhythm is somewhat similar to that of the on-effects. The phase-relations of the alpha wave and the on-effect may be such that the on-effect is obscured. The alpha wave is often distorted when checked by sound stimuli. An alpha individual usually has less alpha rhythm in the vertex and frontal areas than in the occiput. Wherever the alpha is, it is distinguished from other frequencies by being modified when eyes are opened or closed under standard conditions. The alpha in Fig. 1F is the 11-per-sec. rhythm (1st 2 lines). As usual, the on-effect on the occiput record is not as clearly demonstrated as in the vertex, where a 9-per-sec. rhythm is emerging from an alpha rhythm, and is abruptly stopped.

In the non-alpha group, two subjects had cortical patterns which were composed of predominantly fast frequencies. The only response to acoustic stimuli was a checking of the fast frequencies which followed every stimulus (Fig. 1D, 1st line). In another subject fast frequencies appeared upon and during stimulation. In still other non-alpha subjects, marked on- and off-effects were produced without interruptions of the fast frequencies.

Under standard conditions, as briefly defined above, responses to sound stimuli take place in a variable manner. The variability does not appear to depend upon the loudness or on the frequency in the range of tones given. The loud tones were not intense enough to produce a startle reaction. The alpha and non-alpha subjects both show variability of response in the same way. When standard conditions were altered, responses to sound stimuli were also modified. If a subject is made uncertain of the procedure, his "psychological set" is modified (cf. Durup and Fessard, 1935; Knott, 1939; Bakes, 1939). When even an orderly sequence of sounds was given without warning, on-effects often eventually appeared before the stimuli were given. These are interpreted as "anticipatory" on-effects (Fig. 1E, 2nd line). The "psychological set" causes the subject to "anticipate" the stimulus which he thinks may be given.

Some individuals show this anticipation only when random frequencies or irregular time intervals are unexpectedly given. Others would respond with an "anticipatory" on-effect if a series of regular sound sequences were cut short. If the usual duration of the sound was cut short, an off-effect would appear at the time of the usual cessation of stimuli. If it was prolonged, the off-effect occurred before the tone ceased. In other words, many "anticipatory" responses have been produced by altering the frequency and time sequences, but were best brought about by causing the subject himself to be uncertain of the procedure as a whole. The character of the responses, however, did not change.

If the physiological condition of the subject becomes modified as he shifts from the alert state to sleep, and the "psychological set" is maintained, the on-effect becomes more pronounced and more predictable. Contrasting this effect on the responses to sound with the responses of the alpha rhythm to light, four stages may be described as shown in Table 1.

Table 1

	α Rhythm (Eyes Closed, No Sound)	Change in α Rhythm on Opening Eyes	On-Effect to Sound
1. Alertness	present in usual degree	α checks sharply	α check and/or on-effect or no visible effect
2. α-optimum state	maximum for the individual	α check not so clean-cut	on-effects more frequent and definite
3. Drowsiness	reduced and variable, fades out	α rhythm returns (a reversal of stage 1)	higher voltage on-effect
4. Sleep	absent	—	on-effect with return of fast waves [K-complex (see Davis, Davis, Loomis, Harvey, and Hobart, 1939)]

DISCUSSION

A sound-proof room was not used for these experiments because it had previously been observed that people coming into a sound-proof room elsewhere in our laboratories were profoundly affected by the unnatural quiet and the unusual sound of their own voices in such a room. One person remarked that "the utter stillness was a violent stimulus in itself." From these observations, it was felt that the normal state of the individual could best be tested in the electrically shielded, quiet but not sound-proof room. Our auditory stimulation was carried out with eyes closed, which greatly reduced the possibility of eye-blinks or -movements obscuring the on-effects. The distribution, relative magnitude and the shape of the on-effect usually differentiates it clearly from eye artefacts.

Numerous on-effects or "evoked potentials" have been described in animal experiments. It is therefore not surprising to find an on-effect to auditory stimulation in the human cortex. The unexpected feature is rather its diffuse character and the variable conditions of its appearance. Obviously it does not represent the first arrival of afferent impulses in the auditory projection area. It might be thought to correspond to the response of cortical neurons to the first sensory influx, which is apparently the nature of many evoked potentials (Fischer, 1932; Kornmüller, 1937; Bremer, 1937a, b, c; Bishop and O'Leary, 1936). But, according to the analysis by Bartley, O'Leary, and Bishop (1937) of the responses of the optic cortex at least two types of secondary reaction must be recognized, one of short latency, fairly well localized to the immediate sensory projection area, enhanced by strych-

nine and depressed by narcosis, and another slower response of longer latency, spreading more widely through the cortex, depressed by strychnine and resistant to moderate narcosis. It is to this class, which apparently includes the widespread secondary discharge which is enhanced by deep barbiturate narcosis (Derbyshire, Rempel, Forbes, and Lambert, 1936; Forbes and Morison, 1939) and also some of the auditory responses of Bremer, that our present on-effect most probably belongs. The increasing prominence and certainty of appearance of our on-effect with drowsiness (see also Davis, Davis, Loomis, Harvey, and Hobart, 1939) supports this tentative identification, as barbiturate narcosis corresponds rather closely to natural sleep (Bremer, 1937c). The variable conditions of appearance, including the "psychological set" seem to depend on the pre-existing activity or "tone" of the cortex or of the subcortical structures involved in the wide diffusion of the on-effect. The various other secondary effects upon the alpha rhythm and faster frequencies may be interpreted in similar fashion, but our facts are too scanty to warrant further speculation.

CONCLUSION

Acoustic stimuli cause electrical on-effects and off-effects in the waking human brain. Though tones did not always produce visible responses, there appeared to be no difference between alpha and non-alpha types of subjects.

The on-effect, composed of a diphasic and sometimes triphasic wave, was most prominent at the vertex. The first phase was negative. Its latency was about 30 to 40 msec. The total duration of the on-effect was approximately 0.3 sec. or less. The voltage measured from peak to trough ranged from just visible to 100 μV. Frequently there was an off-effect similar to the on-effect, but never as prominent.

A checking of the alpha rhythm was sometimes the only visible response. Fast frequencies were checked in two non-alpha subjects, and caused to appear in a third.

"Anticipatory" on-effects or off-effects appeared at an appropriate interval when a regularly spaced sequence of tones was unexpectedly stopped or prolonged. If a subject was not aware that random sequences of different tones were to be given, or that regular sequences were going to follow irregular sequences, the "anticipatory" on- and off-effects would become more unpredictable. If the "psychological set" remained unaltered in relation to the experiment, but the physiological condition progressed from alertness to sleep, the on-effect would always become more predictable and more prominent.

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ELECTRICAL REACTIONS OF THE HUMAN BRAIN TO AUDITORY STIMULATION DURING SLEEP

H. DAVIS, P. A. DAVIS, A. L. LOOMIS, E. N. HARVEY, AND G. HOBART

From the Department of Physiology, Harvard Medical School, Boston, Mass., and The Loomis Laboratory, Tuxedo, N. Y.

INTRODUCTION

THE SPONTANEOUS electrical activity of the human brain has been described both for the waking state and for sleep by many investigators (see Jasper, 1937, Davis, H., 1939, for references). The modifications of electrical activity as a result of peripheral sensory stimulation in the waking state are slight and have received much less attention. A conspicuous effect in many subjects is the "check," or inhibition, of the 10-cycle "alpha" rhythm which occurs when the eyes are opened. Definite "on-effects," particularly in response to sounds, have been mentioned casually by several investigators and described systematically by one of us (Davis, P. A., 1939, *q.v.* for references).

In sleep one reaction to sensory stimulation is a return of the waking pattern; but three of us have described (Loomis, Harvey, and Hobart, 1938) a more specific disturbance pattern which we designated as the "*K*-complex." The *K*-complex and the waking on-effects are of considerable theoretical importance because of the possibility of identifying them with similar responses of the brains of animals, and thereby coördinating the separate fields of human and of animal investigation. We therefore undertook further investigation of the human *K*-complex in an endeavor to analyze it into its components, and to compare the components with other electrical phenomena in the brains of both man and animals.

METHOD

Twenty-five experiments on sleep were carried out at the Loomis Laboratory in 1938 utilizing the six-channel, ink-writing electroencephalograph and its accessories, described in a previous paper (Loomis, Harvey, and Hobart, 1938). The subjects went to bed either for an afternoon nap or for a full night's sleep. Various types of electrodes (Davis, Davis, Loomis, Harvey, and Hobart, 1939) were employed, including silver, solder, and zinc. Our standard placements were: frontal (at the usual hair-line, 6 cm. to right and left of the midline), central (in the frontal plane of the auditory meatuses, 6 cm. to right and left), occipital (2 cm. above the inion and 5 cm. to right and left), temporal (1 cm. above the tip of the pinna of the external ear). In all cases, recording was by the so-called "monopolar" method. Reference electrodes were placed on one or both ear-lobes or on the mastoid region immediately behind the ears.

RESULTS

On-effects to sounds in the waking state

An earlier series of observations by one of us on the waking on-effect are reported separately (Davis, P. A., 1939). The findings were confirmed in the present experiments. In response to the onset of a steady tone, there is usually a definite diphasic response beginning with a negative wave

(latency 50 to 100 msec.) followed by a slower positive wave (see Fig. 1), in addition to the momentary checking of the alpha rhythm (Fig. 4₁). The diphasic response appears to be a true on-effect. It is widely generalized throughout the cortex and has greater voltage in the central and precentral regions than in the occipital or temporal areas.
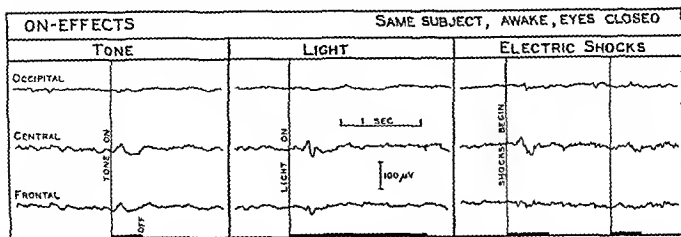
FIG 1 Waking on-effects to tone of 500 cycles at 70 db above threshold, to indirect illumination of room by a 100-watt incandescent lamp, and to electric shocks delivered to left fourth finger The subject is a 37-yr -old man, lying in bed awake, with eyes closed throughout Noise-level in room at position of sleeper's head, principally from ventilator fan, was 35 to 40 db. Intensity of stimulating tone measured also as a noise-level Reference electrode on right mastoid region Scalp electrodes also on right side of head In this and all subsequent figures an upward deflection represents increasing electrical negativity of the scalp relative to the reference electrode on ear or mastoid region

It is unnecessary to repeat the detailed description of the auditory on-effect or the conditions favoring its appearance (see Davis, P.A., 1939), but it is significant that neither the check of the alpha rhythm nor the diphasic on-effect is specific for auditory stimulation. Very similar responses have been obtained from visual and also from electrical stimulation. Figure 1 shows clear on-effects from a subject whose interim record was unusually flat. The minor differences in the shape and latency of the on-effects to light (diffuse illumination from a 100-watt bulb seen through closed eye-lids), tone, and electricity (induction shocks to left fourth finger) tended to be characteristic of the particular form of stimulation, but the distribution over the cortex was the same for all three.

The diphasic on-effect and the modification of the alpha rhythm are obviously both of them generalized secondary reactions of the cortex which may be observed under favorable conditions. They should not be interpreted as equivalent to the immediate and localized responses in a particular sensory area which are seen in experiments on the exposed cortex of animals.

The K-complex in sleep

The response which usually follows auditory stimulation during sleep is much larger and much less variable than the waking on-effect. The response is complex, and its characteristics vary systematically with the

stage of sleep. Figure 2 illustrates the K-complex as it appears in the C stage, recorded simultaneously from six different cortical areas. Shortly after the beginning of the stimulating tone, the scalp becomes electrically negative with respect to the ears by 50 to 100 μV. At about 0.75 sec. the scalp abruptly becomes more positive by 100μV. or more (S in Fig. 2). This major positive wave is followed by a slower return to the original electrical level. Fast waves, often sharp and irregular, sometimes in clear and regular 14-per-sec. rhythm and sometimes in slower 8-per-sec. rhythm (as at F in
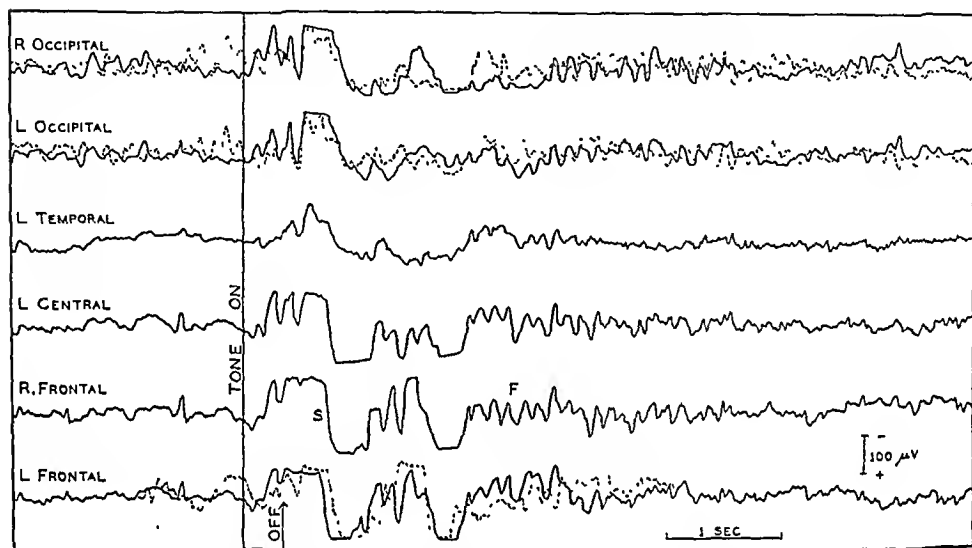
FIG. 2. Typical K-complexes in response to tone during B-C stage of sleep. Afternoon nap, 21-yr.-old man. Tone, 500 cycles at 70 db above threshold. Noise-level as for Fig. 1. Condenser across terminals of loud-speaker to eliminate click at onset of tone in this and subsequent experiments. Reference electrode just above left mastoid. Dotted lines show response at 3 electrodes to a similar stimulus 1.5 min. after the response shown by solid lines. The fast component (F) is prominent and is characteristically 8 per sec. in this subject until deep sleep is reached. Note the artificial flattening of the tops and bottoms of the slow waves (S) by the current-limiting tube in the output circuit (Loomis, Harvey, and Hobart, 1938).

Fig. 2) are superimposed on the slow waves and may persist for several seconds afterward. The abrupt swing from negative to positive usually occurs at about 0.75 sec., but it may be delayed until more than 1 sec. after the onset of the tone. The first slow negative swing is usually preceded in this subject by waves of medium, that is, 6- to 10-per-sec. frequency. The major features of the pattern are usually closely reproduced in successive trials on the same subject, as illustrated by the dotted lines in Fig. 2. Figure 2 also illustrates a definite tendency of the slow-wave sequence to become rhythmic (note particularly the frontal records). A rhythmic activity of the slow component is highly characteristic of deep sleep (cf. also Fig. 3$_{5,6}$).

The distribution of the K-complex over the head follows closely the distribution of the waking on-effect. The voltage is regularly greatest in the central and precentral regions, and nearly as great in the frontal. The disturbance is definitely smaller at the occiput and still smaller in the temporal region. The temporal region, however, always gives a low-voltage record for all features (K-complexes, on-effects, waking alpha rhythm, etc.), perhaps because of the shunting effect of the soft tissues, notably the temporal muscles, external to the scalp. The K-complex may be of higher voltage in the frontal than in the central region, particularly when the major waves are slow and rounded, as in Fig. 4. Very rarely the K-complex is most prominent at the occiput.

A characteristic K-complex is usually produced by even a rather faint tone (20 db above the noise-level of the room) if the sleeper is in the B or C stage. The responses are larger and failures of response are fewer if the tone is loud. The pitch of the tone within the range employed (200 to 3000 cycles) is unimportant except that after a series of tests at one pitch, a shift to a new pitch is rather likely to awaken the sleeper. It is possible to initiate typical K-complexes by turning on a light in the experimental room or by applying mild electric shocks to the subject's finger, but neither of these stimuli are nearly as effective in evoking K-complexes as are sounds.

In one experiment, an effort was made to condition the K-complex to electrical stimulation. Electrical stimulation and sound were combined for a number of trials and then the tone was omitted. The response to the electrical stimulation was not clearly greater than it had been previously. There seemed to be some additive effect between the two types of stimulation, as tone plus electrical stimulation gave a somewhat greater proportion of positive responses than did the tone alone. It is difficult to perform satisfactory experiments of this sort, as the responses vary considerably with the depth of sleep and it is difficult to hold the sleeper in a steady state for a long enough time. Usually he either goes too deeply asleep or else, if stimulated too vigorously or too frequently, he awakens.

Spontaneous K-complexes are common. Sometimes definite causes can be found for them, the commonest being the sleeper's own breath sounds. It is quite amusing to observe the regular appearance of electrical disturbances with each snore, but it interferes seriously with systematic experimentation. For many K-complexes, however, we have found no assignable external cause.

Relation of the K-complex to the stage of sleep

The description of the K-complex thus far has been based on the responses of sleepers in the C stage of sleep. It is in this stage that the K-complex appears most clearly. The genesis of the typical K-complex with the onset and progress of sleep is illustrated in Fig. 3. In this experiment the subject (a 14-year-old boy) was instructed to turn off the tone whenever he heard it, by squeezing a rubber bulb placed in his hand. The waking
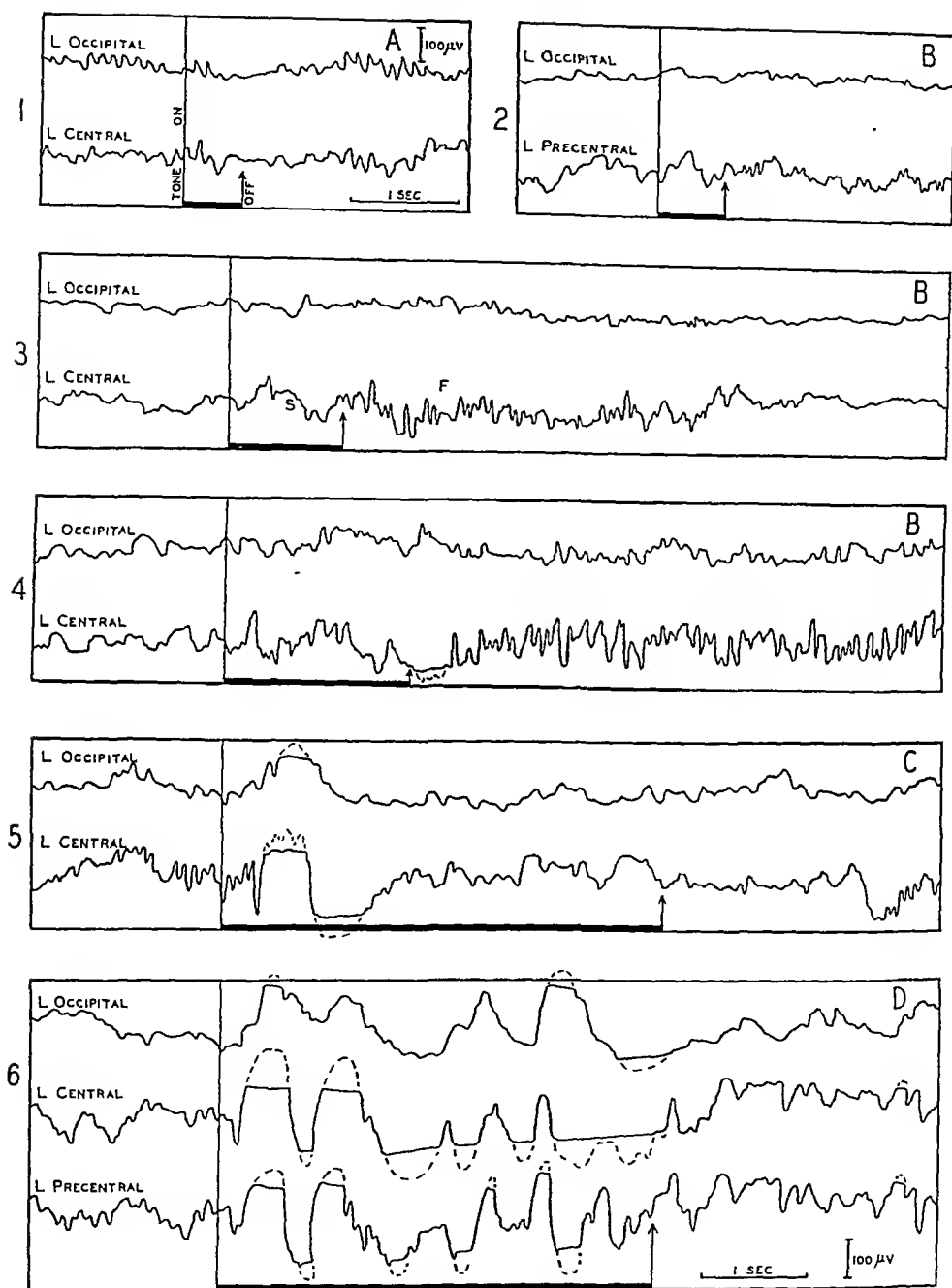
FIG. 3. Responses to tone of 500 cycles during the drowsy state and in sleep. Intensity of tone 70 db in 1, reduced to 55 for 2, 3, 4, 5, and 6. Noise-level about 25 db. Subject is a 14-yr.-old boy, who went to sleep for an afternoon nap. The stages of sleep are indicated by the lines in the upper right-hand corners. (See text for details.) Reference electrode on right mastoid region. Dotted lines indicate the probable approximate course of the potential-changes which are obscured by the current-limiting tube in the output stage.

record shows a strong alpha rhythm, which is almost continuously active (Fig 3_1), and the alpha waves are unusually responsive to auditory signals Perhaps the responsiveness is dependent upon the psychological conditions of the experiment, but this subject invariably showed a transient checking of his alpha rhythm whenever the tone was turned on, irrespective of whether he was instructed to turn it off or to pay no attention to it As the subject became drowsy in this experiment his alpha rhythm became intermittent and returned abruptly when the subject turned off the tone This reaction corresponds closely to the return from a "float" described in a previous paper (Davis, Davis, Loomis, Harvey, and Hobart, 1938)

The subject then passed into the low-voltage B stage of sleep In this stage (Fig 3_2) he continued to turn off the tone, although less promptly, but without any return of his alpha waves The next modification (Fig 3_3) was the appearance, following the tone, of fast waves at the central region It should be clear from the central distribution and varied frequencies of these waves that they are not the usual alpha waves They clearly represent the fast component of the typical K-complex Fig 3_3 also shows the first beginnings of the slow component, "S," during the stimulation It is remarkable that the subject's EEG is now definitely in the "sleep" category, yet he continued to turn off the tone From the beginning of the experiment the tone had been turned on automatically every half minute, and up to this point the subject had not once failed to turn it off In Fig 3_4 the record before stimulation is even more clearly a sleep record, with quite well-developed delta waves, and the K-complex following the stimulus is still better developed, but the subject still squeezed the bulb The subject's reaction time became progressively longer, as illustrated in Fig $3_{1\,2\,3\,4}$ The prolongation correlated well with the changes in electrical pattern, but only when the subject reached the C stage (Fig 3_5), identified by the spontaneous train of 14-per sec waves appearing at the central region, did the subject fail to squeeze the bulb The K-complex was then fully developed

Fig 3_6 shows the further development of the K complex in the D stage of sleep The fast-wave component has become less conspicuous Its waves are slower and rounded and can scarcely be identified because of the high-voltage waves of the slow component, on which the fast waves are superimposed In this D stage the slow (delta) activity becomes so rhythmic and so nearly continuous between stimuli that it is often difficult to determine whether there is or is not a response following a stimulus

In the E stage, which was not reached in this experiment, there is no indication of any modification of the electrical record following even a very strong stimulus It will be recalled that in the E stage the spontaneous delta activity is continuous at high voltage and at frequencies of 1 per sec or less (cf Fig 6) and that in the E stage the trains of 14-per-sec waves are absent

The particular experiment illustrated in Fig 3 is somewhat unusual in

the persistence of the motor reaction, apparently well into sleep. The progressive development of the K-complex is fairly typical, however, if we make allowance for individual characteristics of the K-complexes of various subjects. (For example, the prominent 6- to 8-per-sec. waves in Fig. 2 are characteristic of one particular subject and are quite well developed in two others.) A general rule seems to be perfectly clear. *The slow and the fast components of the K-complexes both resemble closely the delta waves and the fast waves which are characteristic of the spontaneous activity of the particular stage of sleep.* In fact, it now seems clear that most of the characteristics originally selected for the identification of the stages of sleep actually are the characteristics of the spontaneous K-waves, although the fast component of an evoked K-complex corresponds to the stage to which the subject is aroused rather than to the stage in which he was before being stimulated. The low-voltage B stage shows little delta activity, and the slow component of the K-complex is relatively small and fast (Fig. 3_3, 3_4, and Fig. 5). The fast-wave component is also relatively fast or may be represented by a more or less complete return of the waking pattern. The C stage of sleep has well-defined delta activity and prominent trains of 14-per-sec. waves. These trains obviously correspond closely to the fast component of the K-complex. The D stage is characterized by rhythmic delta waves which obviously resemble the rhythmic slow component of the K-complex.

Analysis of the K-complex

We have spoken throughout of the K "complex." We may obviously identify two main components—the slow-wave (delta) component ("S" in Fig. 2, 3, and 5) and the fast-wave component. Either the fast or the slow component may appear alone, or one may be well developed while the other is rudimentary. The two components may be separated experimentally by stimulation at brief intervals (Fig. 4). A second stimulus delivered within 4 or 5 sec. after a previous stimulus rarely evokes the slow component. If the slow component does appear, it is almost always reduced in size, faster in frequency, and often shows an unusually long latency. The fast-wave component, on the other hand, regularly appears following the second stimulus and merges with the fast-wave sequence of the first K-complex, as in Fig. 4.

If a tone is timed to fall 2 or 3 sec. after the major positive swing of a "spontaneous" K-complex, it usually fails to evoke a second slow component. The situation is the same whether the first K-complex is evoked by a known stimulus or is "spontaneous." It appears as if some part of the mechanism necessary for the generation of the slow component were refractory for several seconds after a previous response. The situation in regard to the fast component is quite different. Not only is its appearance not hindered by a previous K-complex, but it is often actually enhanced by it. Also a stimulus may fall in any relation to a "spontaneous" train of 14-per-sec. waves without hindering the appearance of either the slow or fast com-

ponent of the ensuing K-complex The fast component alone does not leave the mechanism refractory for either the fast or the slow component If there is a refractory period involved, it is so short as to be of quite another order of magnitude than that following the slow component

If repeated stimuli are given at intervals of 3 or 4 sec or less, the fast-wave activity tends to become faster and less regular, the waves sharper in contour but lower in voltage The subject often stirs or awakens Only if the subject is so deeply asleep that the fast waves of his K response are
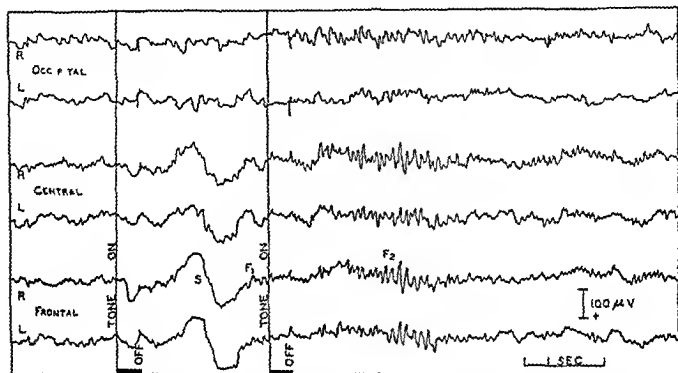
FIG 4 Response to two brief tones at 2000 cycles at 45 db above threshold during B C stage of sleep Noise level 35 to 40 db Subject is a 41 year old woman The slow component S_1, is of the frontal type Note almost complete absence of slow components following the second tone but the greatly increased fast component F_2 Reference electrodes on right ear lobe for lines 1, 3 and 5, left ear lobe for 2, 4, and 6

inconspicuous or are slower than 14 per sec does such repetition of the stimulus fail to shift him to a lighter stage of sleep

Varieties of the slow wave components

The K-complexes of many individuals in light sleep are both characteristic and reproducible In Fig 5 the fast-wave component is present, although poorly developed, and the slow rhythm is typical The sequence begins with a small negative wave whose foot begins about 100 msec after the stimulus Successive waves are higher in voltage and longer in duration, so that no definite frequency can be assigned to them The diagram in Fig 5 is drawn from the average measurements of voltages and times of 13 K-complexes from the same individual The 13 complexes were selected from 25 responses in approximately the same stage of sleep, because it was evident from actual superposition of the original records that they all resembled

one another quite closely. In a few cases, one or both of the first two small waves were absent, and in several the fourth and fifth waves were poorly developed, but all 13 clearly conformed to the same general pattern. The remaining 12 responses showed one, and usually more, of the diagrammatic waves at their appropriate intervals, but they were superimposed on and partially replaced by a somewhat slower sequence of rounder waves of approximately the same voltage (cf. Fig. 4, central and frontal, and Fig. 3_5). The pattern illustrated in Fig. 5 was always most prominent, *i.e.*, highest voltage, at the central region, while the slower, rounder waves of Fig. 4 were best developed in the frontal region. We therefore tentatively designate these two types of slow wave appearing in the K-complex as "central" and
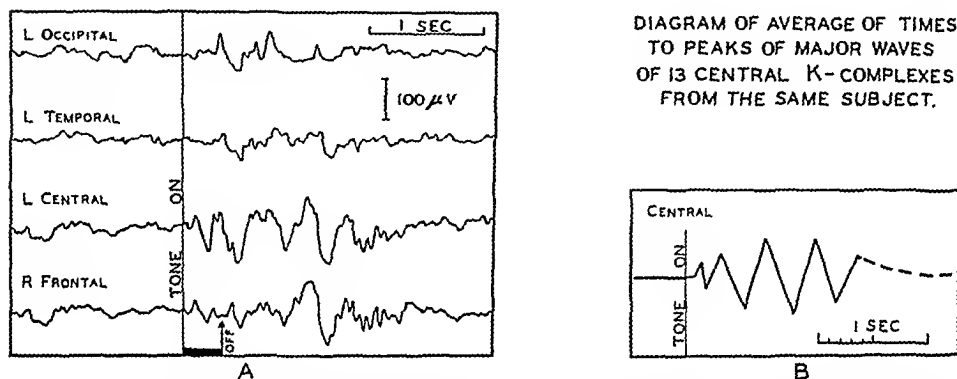
FIG. 5. *A.* Typical K-complex in response to a brief tone of 1000 cycles of 70 db above threshold from a 35-yr.-old man in the B stage of sleep. This response was selected as most typical of the "central" type of slow component. Reference electrodes on left mastoid region for three upper lines, on right mastoid for lowest.
B. A diagram of the composite of 13 "central" K-complexes from this subject.

"frontal," respectively. Some subjects gave a large proportion of pure "central" responses. Others tended to give more of the frontal variety, but more often the responses were mixed in character. In the "mixed" variety, both "central" and "frontal" waves appear both in the central and frontal areas, but the fast are more conspicuous in the record from the central region, and the slower, rounded waves are more prominent in the frontal area.

Intermittent auditory stimuli

The rather prominent 6- to 8-per-sec. "central" rhythm which appeared in some subjects (cf. esp. Fig. 2) and the apparent absence of refractory period for the fast-wave component led us to wonder whether the 6-per-sec. rhythm could be enhanced by stimulating with intermittent sounds (clicks or knocks) at a frequency of 6 per sec. It seemed possible that we might be able to "drive" the waves at frequencies determined by the frequency of stimulation, as Adrian and others have done with the occipital waves in the waking state (Adrian and Matthews, 1934; Loomis, Harvey, and Hobart,

1936) We were unable to produce a corresponding effect in sleep by auditory "flicker" Even rather loud knocks at frequencies near 6 per sec produced no different response from what we regularly observed with steady tones Knocking had, however, a much stronger tendency to awaken the sleeper, perhaps because of its intermittent character or because of its psychological associations

Possible direct-current components of the K-complex

The time-course of the slowest waves and rhythms which appeared in the frontal region raised the question whether our amplifiers, with a time-constant of approximately 0 5 sec, were adequate to record all of the

FIG 6 Comparison of D C record of potential changes (line B) with a simultaneous record from the opposite side taken by the usual condenser coupled amplifier Large delta waves, E stage of sleep A and C were recorded simultaneously The input leads of line C were short circuited automatically 8 5 times per sec (see Davis, Davis, Loomis, Harvey, and Hobart, 1939) Line B is subsequent reconstruction from C Distance of dots in B from a reference line (not shown) is proportional to the excursions in C on opening and closing of the short-circuit

electrical response It seemed possible that there might be a slow component,—a shift of the base-line,—as part of the K-complex which was not being revealed by our condenser-coupled amplifiers We therefore employed the method of interrupting the input to one of our amplifiers (Davis, Davis, Loomis, Harvey, and Hobart, 1939) to determine whether such slow changes existed or not Figure 6 shows how the potential appearing between the input electrodes when the short-circuiting switch is opened is revealed by the height of the square-topped "waves" The course of the potential-change is reconstructed in line B on a scale of sensitivity comparable with that of the usual record Comparison between A, taken from the left side of the head, and line B, reconstructed from the record taken from the right side, shows that the usual method records with considerable fidelity the slow changes of voltage of the E stage of sleep

Interrupted records taken during stimulation by tones could not follow the details of the K-complex, but showed conclusively that there was no slow shift of as much as 50 μV in the electrical base-line associated with the K-complex

sponse to stimulation described by Bishop and his collaborators (Bartley, O'Leary, and Bishop, 1937; Bishop and O'Leary, 1938). On the basis of the time relationships of the waves which he observed, Bishop suggested that his slowest waves represent activation of the mechanism which generates alpha waves in the waking state. Our evidence shows that the slow component of the *K*-complex is probably identical with Forbes's secondary discharge and also with the human waking "on-effect," but that it is clearly distinct from the human alpha waves. It appears therefore that one or another of the various comparisons is at fault. The situation is not disturbing, however, since some of the comparisons rest almost entirely upon similarity in time-relations and it is by no means a necessary conclusion that all electrical waves of similar frequencies represent activity of the same neural mechanism. For example, the "alpha" rhythm at 10 to 12 waves per sec. recorded by Grinker and Serota (1938) from the brains of cats under nembutal anesthesia is probably analogous to the human 14-per-sec. rhythm of sleep and to the "fast" component of the *K*-complex and not to the waking occipital 10-per-sec. alpha rhythm. Incidentally, Grinker and Serota report (*loc. cit.*, p 575) a combination of slow and faster waves from the hypothalamus of the narcotized cat which corresponds closely to our cortical *K*-complex of sleep.

The appearance of *K*-complexes with two recognizable types of slow component, the "central" and the "frontal," suggest that two more or less distinct, but similar, neural systems may be set into action by the sensory stimulus. One system apparently tends to activate primarily the central, the other the frontal cortex, but sometimes the two systems seem to share the control of a given region, and we see the "mixed" type of response. The spread of "central" waves into the frontal region, and *vice versa*, is not a mere electrical artefact. Successive responses which are nearly identical in the precentral region often vary widely in their degree of spread into other regions. The cortical systems which generate the two types of waves apparently interdigitate to a considerable degree, and the extent of the response of each system varies from one test to another. Our experiments offer no indication whether a given neuron always participates in one, and only one, system or whether it may respond now in one and now in another pattern. The problem is exactly similar to the one raised by the alpha waves in the waking state in the precentral region. Sometimes they are 10-per-sec. waves in phase with similar waves at the occiput. At other times there are larger 8- or 9-per-sec. waves which are small or absent at the occiput (cf. Jasper and Andrews, 1938). Sometimes the 8-per-sec. precentral waves are coincident with low-voltage 10-per-sec. occipital waves, and the combination gives a confused record in which neither rhythm appears clearly, but in which both may be identified by careful inspection. Both in the waking and in the sleeping state we encounter a tendency of different regions of the cortex to react in rather similar ways, sometimes closely coördinated, yet at other times independently. When the reactions are independent, the indi-

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DISCUSSION

In the light of the foregoing experimental facts, the K-complex, or "disturbance pattern," of sleep appears as a multiple, diffuse, non-specific, delayed electric response of the brain to external sensory stimulation. It is clearly related to the on-effects seen in the waking state and also to the background of electrical activity characteristic of the stage of sleep in which it occurs. Auditory stimuli have so far proved most effective in evoking it. The effectiveness of auditory stimuli during sleep may be no accident if we consider the general biological function of hearing in the rôle of watchman constantly on guard to signal danger. In sleep the eyes are covered by the eyelids, and vision is in abeyance, but hearing remains active, ready to rouse the sleeper. The electric response is not confined to the auditory projection-area of the temporal region. On the contrary, it appears to be relatively weak over the temporal lobe, and best developed over sensorimotor and frontal areas. Its latency may be considerable, from 0.1 to a full second or even more.

The K-complex consists of at least two components,—the fast and the slow. In deep sleep the "fast" waves may be as slow as 10 or even 8 per sec. and resemble a smooth alpha rhythm. As the sleeper becomes more and more aroused, the "fast" waves become faster and less regular, with no clear 10-per-sec. rhythm. They appear to be more closely related to quick or beta waves than to the waking alpha waves (cf. Davis, Davis, Loomis, Harvey, and Hobart, 1938, p. 34). It seems reasonable to interpret the appearance of the fast component as an indication, and possibly even a measure, of the degree to which the subject has been aroused toward the waking state by the stimulus. Repeated stimuli at short intervals cause an increased (summated) fast component, and the subject is quite likely to awake. In deep sleep (stages D and E), the subject cannot easily be aroused, and the fast component of the K-complex is not at all prominent.

The slow component appears to be a definite response, often reproducible in considerable detail, clearly related to the waking on-effect. Its first waves correspond in latency, polarity, and duration to the waking on-effect. It seems reasonable to interpret the slow component as the waking on-effect modified and prolonged by sleep. Even before the subject goes to sleep, the on-effect becomes more prominent as the subject becomes drowsy. This increase in amplitude is broadly similar to the appearance in the animal brain of a delayed secondary discharge in stimulation of the sciatic nerve under deepening barbiturate anesthesia (Derbyshire, Rempel, Forbes, and Lambert, 1936; Forbes and Morison, 1939).

The slow component of the K-complex is rhythmic. If the same cells respond in the successive waves, we may be sure that their refractory period is shorter than 1 cycle of the rhythm. The slow-rhythm component as a whole, however, shows a refractory period to a second auditory stimulus, although the second stimulus may fall as much as 3 or 4 sec. after the original stimulus. This refractory period is much longer than 1 cycle of the rhythm. We

may infer that it is the avenue of approach to the cortex which is refractory, and not the responding elements themselves (cf. Forbes and Morison, 1939, for a theoretical discussion of the problem of the refractory state in a closely analogous situation). The refractory state may represent a true refractory period, like the long refractory period under dial anesthesia of the pathways of cutaneous sensitivity (Marshall, 1938), or possibly it is a secondary result of partial rousing of the subject, a shift in the general level of activity of the brain, which renders the brain less prone to this particular form of rhythmic activity. The effect might then be compared to the checking of the alpha rhythm by visual stimulation, attention, or an emotional state.

The slow component of the K-complex shows many similarities to the secondary discharge described by Forbes and Morison (1939) in the brain of the cat under barbiturate anesthesia. The resemblance is so close that it seems reasonable to attribute both phenomena to the same type of mechanism and to explain the quantitative differences as due to differences between the brains of the human being and of the cat, between sleep and barbiturate narcosis, and between sound and electrical stimulation of a peripheral nerve. Both phenomena are delayed cortical responses to sensory stimulation. Neither of them appears clearly in the waking state but they become prominent under conditions of depressed cortical activity (sleep and anesthesia) and both increase in size as the depression deepens. Both are generalized responses of the cortex. The latency is considerable in each case. Derbyshire, Rempel, Forbes, and Lambert (1936) state that the usual latency of their secondary discharge is 40 to 80 msec. We have not attempted to measure precisely the latency of the K-complex, because the first wave is often small and indefinite, but it is of the order of 100 msec. If ether anesthesia is given to an animal already deeply anesthetized by pentobarbital sodium (nembutal), the latency of the secondary discharge may be as great as 150 msec. (Forbes and Morison, 1939). The secondary discharge and the slow component of the K-complex both show a long refractory period in the sense that a response appears only after the first of a series of stimuli repeated at a rate of 4 per sec. The secondary discharge is reduced in amplitude unless the interval between stimuli is considerably more than 1 sec. The corresponding refractory period of the K-complex may last for as long as 4 or 5 sec. "Spontaneous" K-complexes, or secondary discharges, render the brain incapable of a second response just as effectively as do responses evoked by sensory stimulation. Forbes and Morison (1939) arrived at a similar conclusion concerning their secondary discharge. Bremer (1937) has emphasized the similarity of the electrical activity of the cat's brain in sleep to that under barbiturate anesthesia, in contrast to its activity under ether anesthesia. If our identification of our slow K-component with Forbes's secondary discharge is correct, it represents an important unification of observations on normal man with data from animal experimentation.

Forbes and Morison suggest that their secondary discharge may be analogous to the third (slowest and latest) component of the complex re-

sponse to stimulation described by Bishop and his collaborators (Bartley, O'Leary, and Bishop, 1937; Bishop and O'Leary, 1938). On the basis of the time relationships of the waves which he observed, Bishop suggested that his slowest waves represent activation of the mechanism which generates alpha waves in the waking state. Our evidence shows that the slow component of the K-complex is probably identical with Forbes's secondary discharge and also with the human waking "on-effect," but that it is clearly distinct from the human alpha waves. It appears therefore that one or another of the various comparisons is at fault. The situation is not disturbing, however, since some of the comparisons rest almost entirely upon similarity in time-relations and it is by no means a necessary conclusion that all electrical waves of similar frequencies represent activity of the same neural mechanism. For example, the "alpha" rhythm at 10 to 12 waves per sec. recorded by Grinker and Serota (1938) from the brains of cats under nembutal anesthesia is probably analogous to the human 14-per-sec. rhythm of sleep and to the "fast" component of the K-complex and not to the waking occipital 10-per-sec. alpha rhythm. Incidentally, Grinker and Serota report (*loc. cit.*, p. 575) a combination of slow and faster waves from the hypothalamus of the narcotized cat which corresponds closely to our cortical K-complex of sleep.

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vidual features characteristic of different regions may be recognized. The idea of independently reacting neural systems occupying the same cortical areas is not new. On-effects and secondary reactions have long been recognized (see Davis, 1939, for references), and in particular Bartley, O'Leary, and Bishop (1937) and Bishop and O'Leary (1938) have clearly distinguished two neural systems in the optic and also in the sensorimotor cortex of the cat (cf. also Adrian, 1936). The present evidence merely emphasizes once more the complexity of the organization and activity of the brain.

Summary

The electric response of the human brain to auditory stimuli during sleep, previously described and designated as the "K-complex," has been investigated in more detail. It is a multiple, diffuse, delayed, non-specific response to external sensory stimulation. Usually a fast component, consisting of a series of more or less regular waves at frequencies of 8 to 16 per sec., is superimposed on a series of slow (delta) waves (Figs. 2, 3, and 5), but either component may appear independently of the other. No shift of electrical base-line ("D.C. component") is associated with the K-complex (p. 501).

Light and electric shocks may elicit K-complexes, but less effectively than do sounds (p. 503). Typical K-complexes may appear "spontaneously" without assignable external cause.

The latency of the K-complex is usually of the order of 100 msec. and may be half a second or more. Both fast and slow components are more prominent in the central and frontal than in the occipital and temporal regions (Figs. 2 and 5). The patterns are simultaneous and broadly similar over all these regions, but characteristic differences between central and frontal types of the slow component are described (p. 507).

The fast component is often identical with the trains ("spindles") of 14-per-sec. waves which are characteristic of the C and D stages of sleep. The appearance of the fast component seems to represent a partial arousal of the sleeper. The slow component develops progressively from the waking "on-effect" (Fig. 1) and increases in amplitude and duration as the subject becomes drowsy and goes to sleep. Both components vary systematically with the stage of sleep (Fig. 3). The waves become slower as sleep deepens, and do not appear in the E stage of sleep. The characteristics of the spontaneous K-complexes are the chief criteria by which the B, C, and D stages were originally identified.

A sound within 3 or 4 sec. after a previous stimulus usually fails to evoke a second slow component. If it succeeds, the second response is delayed and reduced in amplitude. The fast component, on the contrary, appears full sized at all intervals of stimulation and merges with the fast component of the previous complex (Fig. 4).

Forbes and his collaborators have described a "secondary discharge" which appears in the cerebral cortex of the cat under barbiturate anesthesia

following electrical stimulation of the sciatic nerve. The slow component of the K-complex shows the same characteristics as, and is probably strictly analogous to, this secondary discharge (p. 512).

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CONDITIONING OF AFFERENT IMPULSES BY REFLEX DISCHARGES OVER THE DORSAL ROOTS

JAN F. TOENNIES

Laboratories of The Rockefeller Institute for Medical Research, New York

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A DESCRIPTION of the reflex discharge over the dorsal spinal roots, which takes place after stimulation of various sensory nerves, was presented in a previous paper. A general parallelism with the behavior of ventral root reflexes was pointed out, and from a review of the available histological evidence it was concluded that the cells of origin of the fibers carrying the reflexly excited impulses must be in the dorsal root ganglia. The latter view has now been fortified by the more recent evidence of Duncan and Crocker. Nothing was said in the previous paper, however, about what the outgoing impulses do in the periphery. It is now possible to state that the sensory mechanism can be conditioned by them. Experiments in which conditioning takes place will be set forth in the present paper, but before they are described it will be necessary to mention a number of points of contributory significance.

RESULTS

Effect of the temperature of the spinal cord

Recently Barron and Matthews (1939) have made the interesting observation that the size of the reflex discharge into the dorsal roots, evoked by a single afferent volley, is augmented if the spinal cord is cooled. From what is now known, following this observation, it appears likely that in our previous experiments, in which as many as 35 per cent of the alpha fibers were found to be accessible to reflex excitation, the temperature of the spinal cord may have fallen somewhat below normal. But, as will be shown later, this fact does not invalidate the significance of the experiments.

Because of the importance which the factor of temperature has acquired in arguments about dorsal root reflexes, a number of experiments were performed in order to gain additional evidence concerning the extent of the variation that temperature is able to produce.

The size of the reflex was first observed at normal body temperature. The rectal temperatures of 12 cats at rest in a warm room were measured and found to vary between 37.5 and 39.7° C., with an average of 38.5°. This average value was taken as the normal temperature, and the preparations were held as closely as possible to it by means of electric bulbs so arranged as not to radiate upon the back. Under these conditions, when the rectal temperature was normal, the spinal cord temperature, it was to be expected, would also be normal, as no operative procedures were carried out in the region of the spinal column. Decerebrated cats and cats under dial narcosis were examined. After exposure of the saphenous nerve, a pair of electrodes
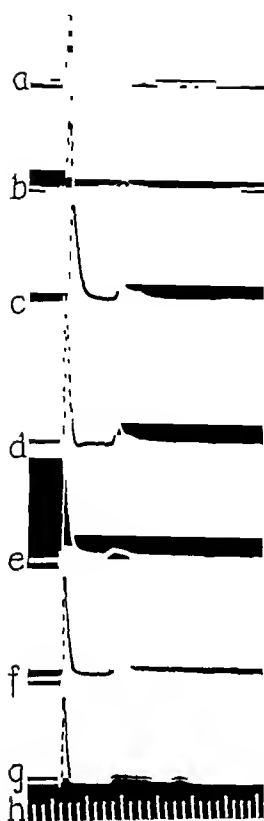
FIG. 1. Saphenous nerve to saphenous nerve reflex in cats at normal temperature (rectal). *a,* decerebrated cat at 39° C. *b,* decerebrated cat at 38.8°. *c,* cat under dial at 38°. *d,* cat under dial at 38°. *e,* decerebrated cat at 38.2°. *f,* the same preparation as *e,* after spinal section; temperature 40.7°. *g,* the same preparation as *e* and *f,* after application of cooling pads to the spine, following removal of the skin. *h,* 1 and 4 msec.

was applied to the end of the nerve in the way usually employed for obtaining a monophasic record of the impulses; and stimulating electrodes were applied central to the leads in order to evoke the afferent volley necessary for the production of a reflex. The records thus contain first, the potential produced by the afferent volley, then that of the reflex discharge. As the afferent volley contained all the alpha fibers, its potential was not subject to variations in size and, therefore, served as a convenient reference potential with which to compare the size of the reflex response.

Examples of records taken at normal temperature are shown in Fig. 1a–e. In every instance a well-marked reflex discharge is visible. Records a and b are taken from decerebrated cats after ample time had been allowed for excretion of the ether used during decerebration; c and d are from cats in dial narcosis. The reflex is relatively larger in the latter, despite the narcosis. Record e is again taken from a decerebrated preparation. This preparation was subsequently made spinal. Following the procedure, the temperature was found to have risen to 40.7° C. without producing much effect on the reflex (Fig. 1f). In order to compare the reflex obtained at the latter temperature with one at a colder temperature, the cord was cooled by denuding the lumbar vertebrae of muscles and applying pads soaked in cold Ringer's solution. After cooling, the reflex discharge increased, particularly in the portion succeeding the first burst of impulses (Fig. 1g). Similar effects were obtained in other preparations. Therefore, a series of experiments was set up in which variation of the temperature of the spinal cord could be accomplished with better control.

The temperature of the cord was measured with a thermocouple (constantan wire in a silver tube) applied through the back deeply into the seventh lumbar segment, on the side opposite to that on which the reflex was produced. Modification of the temperature was effected with the aid of copper tubes through which warmed or cooled water could be circulated. These tubes had a 3 mm. outside diameter and were threaded through the muscles of the back so as to lie on each side of the spinous processes from the lower thorax to the pelvis. This simple method had the advantage that it left the cord undisturbed, but it had the

disadvantage that all parts of the cord would not necessarily be at the same temperature. During cooling the dorsal part of the cord would tend to be colder than the ventral and *vice versa*. And the differential would be greater, the greater the deviation of the temperature of the water in the tubes from body temperature. As the dorsal horns of the cord were between the thermocouple and the cooling tube, it was therefore not to be expected that a series of observations at ascending temperatures would exactly match a series of observations at falling temperatures. The best conditions were those in which the water in the tubes was at a temperature close to that of the region about the thermopile. The temperature of the dorsum of the cord would then be between the observed limits.

Some variation in the effect of temperature was encountered from experiment to experiment; but the results in general were similar. They are fairly represented, as to the course and magnitude of the changes, by the experiment shown in Fig. 2. Record a shows the reflex with the cord temperature at 38.5° C. In size the reflex is comparable to reflexes shown for normal temperatures in Fig. 1. Lowering of the temperature to 30° C. produced a progressive increase in the height of the reflex (Fig. 2b, c, d), especially in the later portion of the discharge. At the normal temperature the reflex discharge, which continues until the end of the record, is in its later portion scarcely discernible at the amplication employed, while at 30° C. the discharge is well-marked throughout the record which is at the same amplification. A return to 38.5° C. restored the original dimensions (Fig. 2e). In the course of further warming, with the water in the tubes at 47° C., the reflex disappeared (Fig. 2f), although the thermocouple recorded only 40° C. This effect was undoubtedly due to the higher temperature in the dorsal horn, occasioned by the temperature gradient, and it disappeared when a stationary temperature of 40° was obtained with a tube temperature of 41° C. The picture at 40° C. then became hardly differentiable from normal (Fig. 2g).

FIG. 2. Effect of changes in the temperature of the spinal cord upon the saphenous nerve to saphenous nerve reflex. *a*, afferent volley and reflex at 38.5°. The spike size of afferent volley is valid for the records *a-g*. *b*, at 36.5°. *c*, at 34°. *d*, at 30°. *e*, rewarmed at 38.5°. *f*, at 40.1° in cord during the temperature increase. *g*, at 40°, 10 min. later at maintained temperature. *h*. 25 times higher amplification, only threshold fibers in the afferent volley (about 2 per cent of the alpha fibers) and the reflex at 38.5° of the spinal cord. *i*, as *h*, but at 31°. *k*, time 1 and 4 msec.

When small afferent volleys were used to produce the reflex, the temperature effect was much more striking. The afferent volley in Fig. 2h, repre-
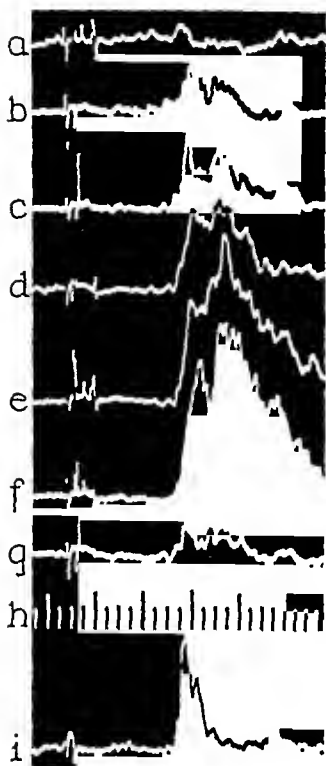
FIG. 3. Effect of changes in the temperature of the spinal cord on the dorsal root reflex in the saphenous nerve produced by a tap on the patellar tendon. *a*, at 38.5°. *b*, at 37.5°. *c*, at 36°. *d*, at 35°. *e*, at 33°. *f*, at 30°. *g*, at 38° rewarmed. *h*, time 1 and 4 msec. *i*, another preparation. Tap on the skin over tibia; reflex recorded on small side branch of saphenous nerve; rectal temperature 38.5°; time as in *h*.

senting about 2 per cent of the alpha fibers, produced a relatively larger reflex at a cord temperature of 38.5° C. than is produced by a larger volley. The area of the reflex potential is approximately equal to that of the afferent volley. But at 31° C. the potential during the first two milliseconds surpassed that of the afferent volley (Fig. 2i).

The effect of temperature was also striking when the reflex into the saphenous nerve was evoked by the afferent impulses arising from a tap on the patellar tendon. The tap was produced with the aid of a device prepared by modifying a small dynamic loud-speaker with a permanent magnet. The paper cone of the loud-speaker was removed from the moving coil and in its place a beveled rubber stopper was attached. In the primary winding of the regular transformer of the loud-speaker, a 1 μF condenser and a thyratron (type 884) were inserted. With this thyratron, which ordinarily served to synchronize electrical excitation with the sweep, the movement of the knee-jerk hammer (*i.e.*, the rubber stopper attached to the moving coil) could similarly be synchronized, as the thyratron controlled the discharge of the condenser (210 volts) through the coil. The secondary winding in the loud-speaker was grounded, in order to reduce the size of the electrical artifact produced in the record.

Figure 3 shows the records that were obtained. Early in the records there appears the electrical disturbance accompanying the tendon tap and this is followed by the potential of the reflex discharge into the saphenous nerve. Starting with a small reflex at a cord temperature of 38.5° C. (Fig. 3a), the reflex rapidly becomes larger as the cord is cooled (Fig. 3b–f). The increase is most rapid between normal and 33° C. Between 33° C. and 30° C. the rate of increase diminishes. On return to a cord temperature of 38° C. the reflex is again reduced (Fig. 3g).

Conditioning of afferent impulses

Afferent impulses were initiated in the saphenous nerve by a gentle tap on the skin of the leg. The tap was applied with the same device that was used for eliciting the knee-jerk, except that a flat piece of felt-covered wood,

5×2 cm. in size, was attached to the moving coil of the loud-speaker. The course of the free swing of the coil and its attachments, recorded by a method which translated the movement into potential change, is shown in Fig. 4a. The total duration of the swing including the recoil was 12 msec. When the skin was in contact with the surface of the stimulator during its movement the period was prolonged but slightly, and the principal effect of the contact was to reduce the amplitude of the swing. The strength of the tap was such that when it was applied to the back of the hand, it produced a sensation of mild pressure.

When the tap was applied to the skin over the tibia of the cat there resulted a discharge of impulses which lasted some 20 msec. and was characterized by a series of crests. The form of the discharge as recorded monophasically from the peripheral end of the cut saphenous nerve is shown in Fig. 4b. If the saphenous nerve fiber connections between the skin and the central nervous system were left intact and a branch of the saphenous nerve was prepared for leading, it was found that this physiologically excited discharge from the endings produced a reflex. Figure 3i shows a reflex produced in this manner in a cat, the rectal temperature of which was $38.5°$ C.

In order to ascertain whether interference with the afferent impulses is occasioned by the reflex discharge it is necessary to record the impulses from the saphenous nerve soon after the passage of the reflex discharge toward the periphery. From the nature of the experiment it is evident that the continuity of the nerve fibers between the skin and the spinal cord must be maintained. Diphasic leads from an exposed portion of the nerve are necessary and the situation is one which presents serious technical difficulties.

Because of the length of the discharges from the periphery diphasic recording is not suitable, for the evidence for the number of fibers involved is obscured by the fact that the second phase of the diphasic potential in some fibers tends to cancel out the first phase of the potential in others. Furthermore the difficulty, which is great enough with respect to the discharge in isolation, is greatly augmented when the latter is opposed to the reflex discharge. The reflex evoked by a single shock applied to the homolateral sciatic nerve lasts about 20 msec. When its impulses precede the afferent impulses, which have a similar duration, by a short enough interval to condition them, the two trains overlap in the nerve and the resulting potential picture is one in which it is almost impossible to determine with accuracy whether there is a deficit of impulses in the centrally directed stream as compared with the unconditioned stream.

A second source of confusion making difficult the interpretation of records of a nerve that has both peripheral and central connections with the body is in the interfering leads from the nerve at its points of junction of the freed portion with the body tissue. One electrode on the freed portion of the nerve may lead by way of the nerve and the body tissue the events occurring at the junction on the opposite side of the paired electrode. It is possible to reduce this source of error by freeing the nerve for a long distance, so that

the resistance of the nerve from the electrodes to the body may be made much higher than that in the interelectrode region. But even this precaution is not sufficient to avoid distortions which will interfere with measurements of small details in the potential. In order to eliminate completely unwanted leads by way of the body tissues, the leg was amputated just below the knee, leaving the saphenous nerve as the only connection between the leg and the rest of the body at the time the leads were made.

In order to preserve the excitability of the sensory endings in the amputated leg, the blood supply was maintained as long as possible. First the skin was severed. Then the muscles were divided and the fibula and tibia cut without disturbance of the major blood vessels. Only after the leg had been safely supported and mounted ready for stimulation of the skin by the tap from the loud-speaker system and the electrodes had been put in place on the nerve, were the blood vessels finally tied and cut. There then remained a period of 20 to 30 minutes during which the experimental manipulations could be carried out before asphyxiation made the sensory endings inexcitable.

The difficulty arising from the length of the discharges was met by cooling the nerve in the stretch from which the leads were made. The electrodes were inserted in a groove within a transparent bakelite block and provision was made for holding the block so that the free portion of the nerve would lie in the groove. When the nerve was in place, the groove was sealed with cotton wool soaked in liquid paraffin. Through channels about the groove within the bakelite block, water at any desired temperature could be pumped, and the temperature of the nerve thereby controlled. The cooled region of the nerve was 38 mm. long and the electrodes were 8 mm. from the ends. Therefore, the interpolar distance was 22 mm. From the nature of the arrangement it is apparent that impulses would enter the electrode block from the central end through fibers at body temperature, and from the peripheral end through fibers at room temperature (25° C.)

Cooling prolongs the refractory period. Beginning with the normal refractory period of about 0.5 msec. at normal body temperature, the refractory period increases as the temperature falls, slowly at first and then more rapidly, so that below 10° C. it comes to be between 20 and 30 msec. (Fig. 6m). This prolongation of the refractory period causes a simplification of the potential picture of the discharges. As the discharges are repetitive, the later impulses in the train fall within the refractory periods of the earlier ones and are blocked. How the blocking occurs among impulses coming from the sensory endings can be seen in parts c, d, and e of Fig. 4. In record e taken from the nerve cooled to 10° C. the picture is made up mostly of potentials belonging to the first component of the discharge. The latter now appears prolonged because of the increased temporal dispersion, and augmented in size because of the increase in area of the individual spikes.

That the impulses belonging to the reflex are also repetitive follows from their similar behavior in cooled nerve. Figure 5 shows records in the saphe-

nous nerve of reflexly excited impulses which have entered the cooling electrode from nerves at body temperature. A comparison of the records obtained at 28° C. with those obtained at 15° C. reveals a deficit in the later components in the cooler preparation, because of prolongation of the refractory period. A second consequence of the long refractory period is that the centrifugal discharge can block the centripetal discharge in its course
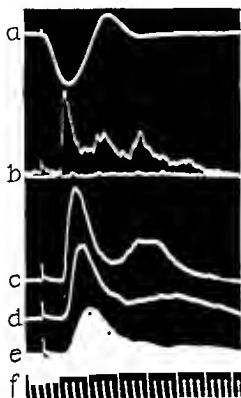
FIG 4 *a*, course of the deflection of the loud-speaker coil used for production of mechanical stimuli *b*, afferent impulses recorded monophasically from the peripheral end of the saphenous nerve (at 25°) as produced by a tap on the skin over the tibia The lower line shows the level of spontaneous impulses and of noise. *c*, as *b*, except that the temperature within the electrode holder was lowered to 15°. *d*, as *b* and *c*, but at 12°, *e*, at 10°, *f*, time 1 and 4 msec
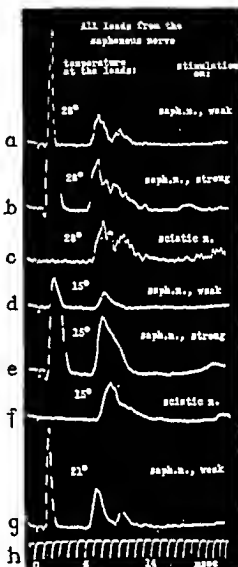
FIG 5 Repetition of impulses in the dorsal root reflex discharge The later parts of the reflex are blocked when the saphenous nerve inside the lead electrode is cooled to 15°.

through the nerve even when the one precedes the other by a considerable interval.

After these preliminary considerations it is now possible to follow the course of a conditioning experiment, such as that shown in Fig. 6. Records *a* and *b* were taken when the temperature within the electrode was 25° C. The first record, *a*, shows the afferent impulses. Two major sharp peaks downward complicated by secondary peaks are to be noted. At the arrow the

reflex evoked by these impulses appears. Its initiation is marked by two sharp peaks upward, because the impulses are running in the direction opposite to those which constitute the early part of the sequence. Record b
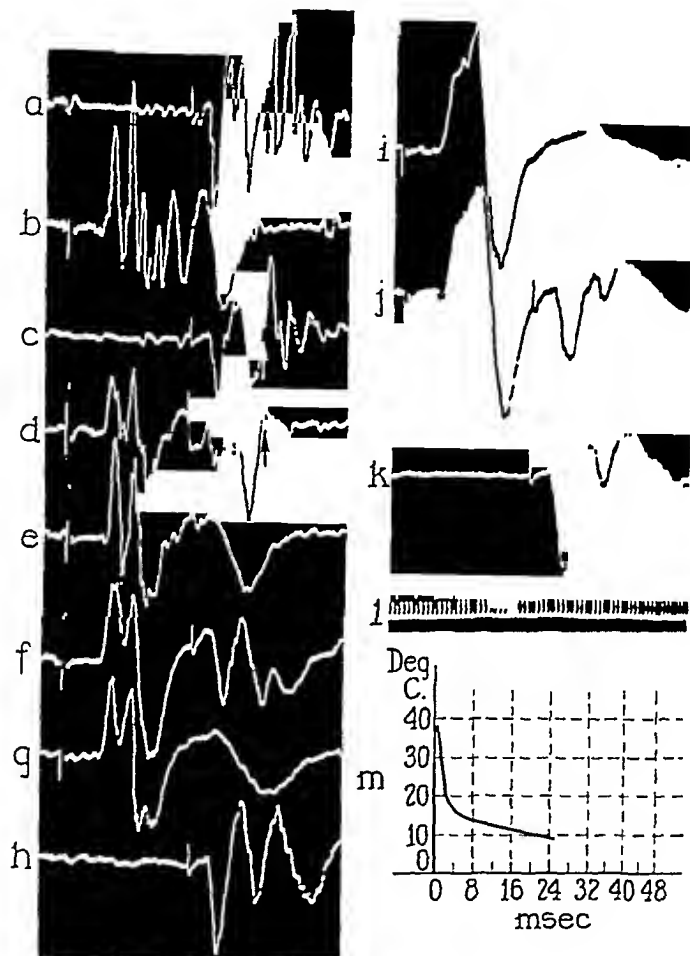
FIG. 6. Afferent discharges from sensory endings conditioned by dorsal root reflex discharges. Cat under dial. All leads diphasic from the uncut saphenous nerve. a, afferent impulses produced by tap on the skin over the tibia. b, reflex produced by a single electrical shock to the sciatic nerve. Records a and b taken at temperature of 25° within the perfused bakelite electrode with which the leads were made. c, tap response at 19°. d, sciatic nerve to saphenous nerve reflex, and the tap response as in c, at 19°. e, sciatic reflex alone at 19°. f, sciatic reflex and tap response at 13°. g, sciatic reflex alone at 13°. h, tap response alone at 13°. i, sciatic reflex at 10°. j, sciatic reflex and tap response at 10°. k, tap response at 10°. l, time 1 and 4 msec. m, total refractoriness of alpha fibers at different temperatures.

shows the reflex produced by a single shock applied to the homolateral sciatic nerve. The component at the end of the series, which is longer than the previous components, is the one travelling in delta fibers. It may be

identified in subsequent records at lower temperatur .... and prolonged

When the discharges shown in records 6a and 6b were pitted one against the other, the record of the conditioning effect of the efferent discharge on the afferent discharge was too complex to be read and it is not shown. Uncertainty still attends the c, d, e series taken at 19°. But attention may be drawn to one feature of record d, *i.e.*, that the reflex produced by the afferent impulses from the skin has disappeared. The disappearance is attributable to inhibition of the reflex in the spinal cord, as a result of the conditioning effect of the activity set up there by the impulses that had previously arrived from the sciatic nerve.

However, in the f, g, h series taken at 13° C. a reading is possible. The potentials of both the discharges are now simplified and the delta component of the sciatic to saphenous reflex picture (Record g) is sufficiently delayed not to interfere with the measurement of the size of the first component of the sensory discharge. Record f shows that the first component is considerably reduced as compared with the control in Fig. h. Also careful comparison of an algebraic summation of g and h with the record of the potentials of the opposed discharges in f indicates that there is also a deficit in the second component of the afferent discharge.

On cooling the nerve in the electrode chamber to 10° C. the potentials were further simplified. The delta elevation in the sciatic-saphenous reflex (i) is now so delayed and flattened as not to interfere with either of the two components that make up the sensory discharge (k). It is obvious from record j that both components of the sensory discharge are reduced when the latter is conditioned. The reduction is well outside of the variation in the pictures of the sensory impulses obtained in a series of tests of the discharges in isolation.
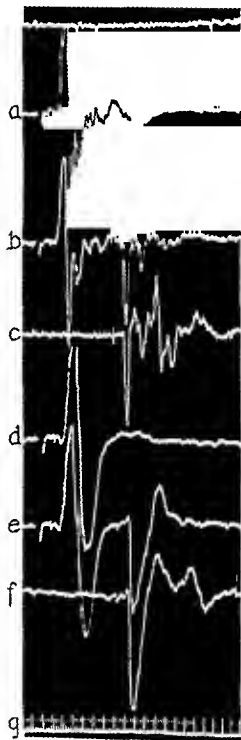
Fig. 7. Afferent discharges from sensory endings conditioned by dorsal root reflex discharges. Cat under dial. *a, b,* and *c,* with nerve at 15°. *a,* sciatic to saphenous reflex. *b,* sensory discharge preceded by a sciatic to saphenous reflex. *c,* sensory discharge. *d, e,* and *f,* with nerve at 10°. *d,* reflex. *e,* sensory discharge preceded by reflex. *f,* sensory discharge. *g,* time 1 and 4 msec.

Conditioning of sensory impulses by an antecedent reflex discharge is also shown in Fig. 7. The records from the nerve at both 15° and 10° C. are favorable for the measurement of the deficits, as the delta elevation of the sciatic-saphaneous reflex is small at 15° C. and nearly invisible at 10° (Records a and d). Comparison of c with b shows that in b there is a deficit in both the components of the sensory discharge. The reflex recorded in c has been inhibited in b. When the nerve was cooled to 10° C., the record of the impulses from the sensory endings was reduced to a single component (f). This component was decreased by a preceding reflex discharge (e).

DISCUSSION

All the experiments which were brought to a successful conclusion were in agreement in showing that afferent impulses arising from the skin as the result of mild mechanical stimulation, imitating the kind of stimulation that occurs hundreds of times a day in ordinary experience, are conditioned if they are preceded at an appropriate interval by a reflex discharge issuing from the spinal cord over the dorsal roots. A deficit in the quota of impulses in the discharge occurs, attributable either to failure of the impulses to be set up in the endings or to blockade in the fibers. In the several experiments the leading impulses in the reflex reached the periphery 4 to 13 msec. ahead of the slap and the first impulses were followed by later ones. From what is known of the effects of backfiring single volleys into the skin over sensory fibers it is certain that the number of impulses set up in the endings by the slap would be decreased at these intervals. In so far as impulses are set up in any of the fibers that had been occupied by the reflex, they would be blocked by the prolonged refractory period in the cooled region.

Because of the uncertainty about the number of impulses set up by the slap, the conditioning revealed in the experiments is susceptible of two interpretations. The first one depends upon the eventuality that the deficit of impulses observed arose from refractoriness or subnormality of the endings following upon participation of the fiber in the reflex or from prolonged refractoriness in the cooled segment of the nerve blocking impulses actually set up. In this event the observations would mean that sensory impulses and the reflex discharge are carried in the same fibers. The second interpretation demands the assumption of a special system of centrifugally conducting dorsal root fibers capable, when active, of conditioning sensory endings. Further work will be necessary before a decision can be made between the two possibilities. The only conclusion permissible at present is that reflexly evoked impulses have an intimate relationship to the sensory mechanism.

The experiments were carried out before the great importance of the temperature of the spinal cord as a determinant of the size of the reflex was realized; and as special precautions to maintain the temperature of the cord were not taken, the temperatures in all probability dropped somewhat below normal. This drop would augment the reflex and therefore

facilitate conditioning, but the significance of the fact that conditioning occurs would not be obscured thereby However, no conclusion is possible about the extent to which central conditioning of peripheral endings enters into the physiology of sensory mechanisms

SUMMARY

When the action potential of the sensory impulses conducted through the saphenous nerve of the cat following a gentle tap on the skin is recorded from the side of the nerve, it is found that a deficit in the total number of impulses occurs if the train is immediately preceded by a reflex discharge through the nerve from the spinal cord

The experiments conducted in the course of the present investigation permit the mention of a number of characteristics of the reflex discharge by way of the dorsal roots, not included in the previous report

    a The reflex discharge into the individual fibers is repetitive

    b The reflex can be evoked by physiologically selected afferent impulses, as those set up by a tap on the skin or to the patellar tendon

    c As shown by Barron and Matthews, the size of the reflex is greatly augmented if the cord is cooled However, well defined reflexes are regularly present when the cord is at a normal temperature

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SPINAL PATHWAYS FOR NICTITATING MEMBRANE REFLEXES IN THE CAT

S. HOWARD ARMSTRONG, JR., AND DAVID McK. RIOCH

From the Department of Anatomy, Harvard Medical School, Boston

THE NICTITATING membrane (n.m.) of the cat has, in recent years, taken a place beside the cardiovascular system as a convenient indicator in the elucidation of autonomic function. In addition to the n.m.'s use in analysis of humoral, synaptic and myoneural junctional phenomena, its reflex activity has been studied from several standpoints. Rosenblueth and Bard (1932) have reviewed the structure and innervation of the n.m. The characteristic forms of its reflex responses to various types of peripheral afferent stimuli have been described in detail by Rosenblueth and Schwartz (1935), with particular reference to excitatory rebound, after-discharge and absence of parallel sign with concomitant vasomotor reflexes. The peripheral afferent nerves giving these responses have been investigated by Acheson, Rosenblueth and Partington (1936). Morison and Rioch (1937) studied the influence of forebrain and midbrain mechanisms on the reflex responses of the n.m. under urethane anaesthesia by methods of cortical and subcortical stimulation and extirpation. They concluded that at least three supraspinal areas contributed to the excitatory components and two or more to the inhibitory components of the n.m. reflexes. No responses were obtained following transection of the brain stem caudal to the midbrain. They further confirmed the lack of parallelism in sign and form between vasomotor and n.m. responses to identical stimuli. Watkins (1938) has extended this observation in taking simultaneous records of B. P. and n.m. changes during distention of hollow viscera.

In connection with these studies the spinal pathways of n.m. reflexes become of interest, and particularly so since Ranson and his coworkers (Ranson and von Hess, 1915; Ranson, 1916; Ranson and Billingsley, 1916a and b) showed that the spinal paths of the depressor and pressor reflexes, respiratory reflexes and "pain" were not localized in the same parts of the spinal cord.

METHODS

Adult cats were used throughout. In chronic preparations cord lesions were made with fine pointed scissors following aseptic laminectomy; dural slits were left open and wounds were closed with silk in layers. No wound infections were encountered. All experiments were performed under urethane anaesthesia (1.3–1.8 g. per kg.) injected intravenously after preliminary brief etherization. N.m. movements were recorded isotonically by a light, counterbalanced heart lever with a magnification of 13 to 18 times. In some experiments respiration was recorded by a tambour attached to a side arm tube of the tracheal cannula, and blood pressure tracings were made by a mercury manometer connected with a carotid cannula. Both sciatic nerves and a bundle of brachial nerves, prepared after the method of Ranson to equal roughly the cross section area of a single sciatic nerve, were used as afferents. Thus in each experiment, n.m. tracings were obtained from levels both caudal and rostral to spinal segments wherein lesions were made. Buried shielded electrodes were

applied to nerves following peripheral crushing In a few instances the hind legs were denervated, this procedure was found not to affect results significantly and therefore was not followed routinely Stimuli were induction shocks at tetanizing frequency from a Harvard inductorium with 3 v in the primary circuit

In acute experiments cord sections were made immediately after laminectomy Kymograph tracings were taken immediately before and up to 4 hr after sections, the animals were kept under light anaesthesia throughout by small supplementary intravenous urethane injections In two experiments the carotid sinuses were denervated and the vagi were cut bilaterally with consequent augmentation of those n m responses still present in the preparation but no restoration of those responses already abolished by cord lesions above the level of stimulation Hence these procedures were routinely omitted The level of the lesion was determined by autopsy following each experiment, and the extent of the lesion was checked by either freehand or serial sections of involved cord segments fixed in formalin

RESULTS

1 *Total section* In three cats, of which two were acute and one a six-day post-operative preparation, complete cord section at L3, T9, and T10 respectively abolished n m response on sciatic stimulation, brachial control readings showed responses within normal limits

2 *Posterior column section* The posterior columns were cut in five cats; in three of these the lesion was limited to the posterior columns alone, and
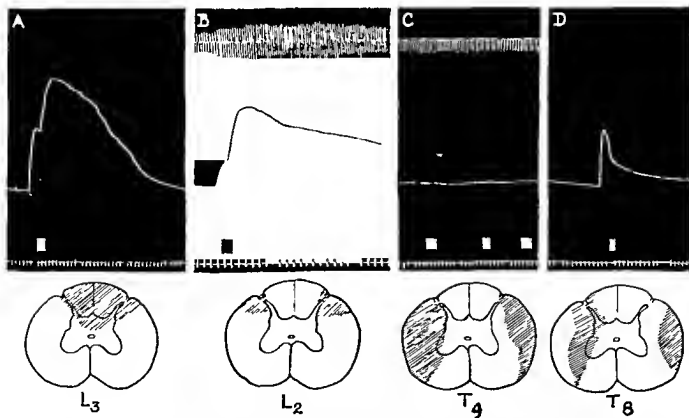
FIG 1 Nictitating membrane responses to sciatic stimulation in cats with bilateral lesions of the spinal cord Records reading from below upward, show Time, in 5 second intervals, Signal marker of stimulation, Nictitating membrane record, (in B and C) Respiration record The diagrams below each record are representations of the extent of the lesion as determined by serial sections

A Section of posterior funiculi at L3 Left sciatic stimulated, 7 cm coil distance

B Lissauer's tracts at L2 Left sciatic stimulated, 6 cm coil distance

C Lateral columns, rt complete, lt outer half, at T9 Left sciatic stimulated, 12, 10, and 4 cm coil distance respectively (cat 39, Table 1)

D Outer half rt and inner half lt lateral column at L3 Left sciatic stimulated, 4 cm coil distance (cat 4a, Table 1)

placed at C6, T8, and L3 respectively; in the fourth and fifth the posterior columns were cut in the course of posterior hemisection at T6 and L3. In all cats autopsies showed lesions to be complete. In the instances of T8 and L3 lesions, the n.m. responses from stimulation both above and below were

Table 1. Nictitating membrane responses evoked by sciatic and brachial stimulation after bilateral lesions of the lateral columns of the spinal cord in the lower thoracic region.

Cat number	Level and lesion	Sciatic stimulation			Brachial stimulation			Time post-op.
		coil distance	rise	re-bound	coil distance	rise	re-bound	
9a	T8, lat. cols. complete. Small hemor-rhage in ant. cols	2 cm.	0	0	5 cm.	+ + + +	+ +	1½ hrs.
39	T9, lat. cols. rt. com-plete; lt. outer half only*	4 cm.	0	0	4 cm.	+ + +	+	8 days
4a	T8, lat. cols. rt. outer half cut; left inner half cut, outer half intact.**	4 cm.	+ +	0	no record			2 hrs.
200	L3, lat. cols. complete	6 cm.	0	0	6 cm.	+ + + +	+	5 days
7a	L2, lat. and ant. cols. complete	1 cm.	0	0	6 cm.	+ + + +	+ +	1 hr.
46	L3, lat. cols. rt. and lt. incomplete	6 cm.	+ + + +	0	6 cm.	+ + + +	+	7 days.

* Figure 1, C.   ** Figure 1, D.

normal in initial amplitude and in the extent of the excitatory rebound (Fig. 1A). The n.m. response following posterior column section at C6 consisted entirely in excitatory rebound for the first four hours, but thereafter resumed its preoperative contour.

3. *Lissauer's tract:* The apices of both posterior horns and the adjoining tracts of Lissauer were cut bilaterally in five cats. In two cases the lesions were limited to these areas alone and placed at the same level, T9 and L3 respectively. In a third Lissauer's tracts were sectioned on one side at L2 and on the opposite side at T8. In the two remaining cats the tracts were cut in the course of the posterior hemisections at T6 and L3 mentioned in

the preceding paragraph Autopsies showed complete lesions In all cats strong n m contraction with normal rebound followed sciatic stimulation (Fig 1B)

4 *Lateral columns* The lateral column was cut on both sides in the same segment in six cats, and the n m reflexes were tested at intervals varying between one hour and eight days post-operatively The results are

Table 2 Nictitating membrane responses evoked by sciatic and brachial stimulation after hemi section of the spinal cord in the upper lumbar and contralaterally in the mid thoracic regions

Cat num ber	Level and lesions	Sciatic stimulation			Brachial stimulation			Time post op
		coil dis tance and side	rise	rebound	coil dis tance	rise	rebound	
202	L3 lt hemisec T8 rt hemisec	Rt 6 cm Lt 6 cm	0 0	0 0	6 cm	+++	+	left hemi six days before exp rt hemi during exp
203	L3 lt hemisec T6 rt hemisec	Rt 3 cm Lt 3 cm	0 0	0 0	6 cm	+++	++	2 hrs
14	L3 lt hemisec T7 rt hemisec	Lt 4 cm Rt 4 cm	0 0	0 0	6 cm	+++	++	2 hrs
15	L2 rt lat col only T8 lt hemi- section	Lt 6 cm Rt 5 cm	0 0	0 0	6 cm	+++	+	6 days

summarized in Table 1, and show that when the lesions of the lateral columns were complete there was no response of the n m to sciatic stimulation, though normal reflexes were elicited from the brachial nerves

5 *Crossing of pathways* Single hemisections were made in three cats at T10, T12, and L3 respectively N m tracings were made one to two hours post operatively and showed responses normal and equal in extent and rebound on stimulation of either sciatic To determine the lower limit of crossing, the cord was exposed from L2 down to the cauda equina in one cat Successive hemisections on the left were made in each segment throughout the lumbar cord, after each hemisection n m tracings were taken from each sciatic After the second hemisection (L3) rebound disappeared on both sides, but responses equal in extent and approximately one-third that of the pre-operative reflexes could be obtained from either sciatic A single hemisection placed at L1 on the right, *i e*, one segment above and

contralateral to the original hemisection, abolished sciatic n.m. responses; brachial stimulation was still effective, but the responses were about one-half as strong as in the pre-operative control period and there was no rebound. Autopsy showed all lesions to be either complete hemisections or to be slightly more than complete.
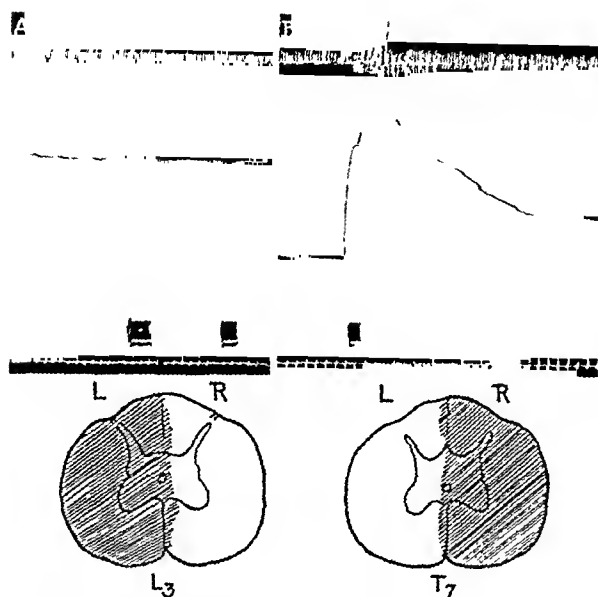
FIG. 2. Nictitating membrane responses to sciatic and brachial stimulation in cats with hemisection of the spinal cord in the upper lumbar and contralateral hemisection in the mid thoracic region. Records as in Fig. 1. Diagrams show extent of lesion at L3 and T7 respectively. (Cat 14, Table 2).

A. Sciatic stimulation, left and right respectively, 4 cm. coil distance.

B. Brachial stimulation, 6 cm. coil distance.

To determine whether crossing occurs in the upper lumbar and lower thoracic cord, above the level of entry of the sciatic fibers, contralateral hemisections at upper and lower levels of this region were studied in four cats. The results are summarized in Table 2, and show that one hemisection of the spinal cord at L3 or L2 and a second, contralateral, hemisection at T6 to T8 abolish all n.m. responses to stimulation of either sciatic, though the responses to brachial stimulation remain essentially normal (Fig. 2).

In five cats hemisections were made between C5 and C6, together with contralateral lower hemisections, and records were taken from both membranes. All five cats were operated four or more days before the final experiments. The results were inconstant. In two cases essentially normal reflexes were obtained in both membranes on sciatic stimulation. In three cases the membrane ipsilateral to the cervical hemisection responded normally or without rebound, whereas the contralateral n.m. responded poorly or not at all. In all cases the responses to brachial stimulation were normal and equal.

DISCUSSION

Using the sciatics as afferents, Ranson and coworkers (Ranson and von Hess, 1915; Ranson, 1916; Ranson and Billingsley, 1916a and b) have mapped the lower thoracic and upper lumbar spinal pathways for the vasomotor autonomic reflexes in the cat. They found the pressor afferent pathway from a given sciatic to lie bilaterally in Lissauer's tract, with conduction somewhat better ipsilaterally; the depressor pathway bilaterally in

the anterior part of the lateral columns, with conduction somewhat better contralaterally. Reflex acceleration of respiration was reduced but not abolished by bilateral section of either or both of these regions, or by posterior hemisection. These workers, however, were unable to demonstrate any hypalgesia to "conscious pain," as evidenced by struggle and cry in unanaesthetized chronic preparations, with either posterior hemisection, lateral hemisection, bilateral sections of lateral columns, or two hemisections placed on opposite sides of the cord a few segments apart. The degree of crossing of the vasomotor reflexes above the level of sciatic entry was not specifically investigated.

Harper and McSwiney (1937) have found that afferent pathways for reflex pupillary dilatation on stimulation of either hypogastrics or intercostals lie both ipsilaterally and contralaterally in the lateral columns, with all crossing in the segment of entry. In the experiments reported here it has been demonstrated that normal n.m. reflexes are evoked by stimulation of either sciatic after section of the posterior funiculi (p. 528) or after bilateral destruction of the apices of the posterior horns together with Lissauer's tracts (p. 528). Complete section of the lateral columns on both sides abolished the responses (p. 529). Moreover, in cat 4a (Table 1, and Fig. 1D) the superficial half of one lateral column was preserved and the n.m. responses were present, though reduced, from both sciatics. It may be concluded that the afferent pathways in the spinal cord lie in the lateral columns, the fibers being probably diffusely distributed in the superficial parts of these columns. Since equal responses were obtained from either sciatic following hemisection of the cord (p. 529), it would appear that conduction is equal in both crossed and uncrossed pathways. The abolition of responses by two hemisections, one at L2 or L3 and the other contralaterally at T4 to T8 (p. 529) indicates further that the crossing occurs within one or two segments from the level of entry and that there is no further crossing in the upper lumbar or lower thoracic portions of the cord.

In these respects the lumbar and lower thoracic spinal course of the afferent path for the n.m. reflexes is similar to that described by Harper and McSwiney (1937) for the pupillodilator reflexes. It differs from the afferent path for the vasodepressor reflexes as described by Ranson et al. in two respects: (i). since ipsi- and contralateral conduction are approximately equal by the methods used; and (ii). the pathway is apparently diffusely distributed in the superficial half of the lateral columns.

The experiments on combined, contralateral hemisections at L3 and C5 or C6 (p. 530) indicate that the spino-spinal afferent paths from both above and below the level of motor outflow to the n.m. (Langley, 1895) cross below C6 and above T8 respectively. It seems probable that this crossing is at the level of the motor outflow itself. Since Morison and Rioch (1937) found that the rebound phenomenon in the reflexes was dependent on the integrity of certain forebrain mechanisms, and that no reflexes were obtained following transections below the medulla, it would seem likely that the

reflexes dealt with here are spinal reflexes, facilitated by forebrain mechanisms the efferents of which cross below the level of C6. The other possible explanation for the phenomena under consideration would be that the reflex arc includes forebrain mechanisms and that the afferents cross to a large extent between T6 and C6. This seems less probable.

Langworthy and Richter (1930) evoked strong skin galvanic responses by stimulation of the posterior column nuclei, and Cannon and Rosenblueth (personal communication) obtained large n.m. responses by stimulation of the central ends of the cut, isolated posterior funiculi in the upper cervical region. In view of these observations it is of interest that section of the posterior funiculi in the thoracic or cervical levels was without effect on the n.m. reflexes in either form or extent in the present experiments.

Summary and Conclusions

The afferent pathways through the spinal cord for reflexes of the nictitating membrane (n.m.) in cats were studied by recording the n.m. responses to sciatic and brachial stimulation under urethane anaesthesia after localized lesions of the spinal cord.

It is concluded that the pathways lie in the lateral columns of the cord and are approximately equally crossed and uncrossed. Crossing takes place at the level of entry of the sciatic posterior roots and no further crossing can be demonstrated between this and the lower limit of the level of the sympathetic outflow to the n.m. Crossing again occurs at the n.m. center in the thoracic cord both for spino-spinal afferents from the brachial and sciatic nerves and probably for forebrain efferent mechanisms.

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VAGAL INHIBITION OF INSPIRATION, AND ACCOMPANYING CHANGES OF RESPIRATORY RHYTHM

T. E. BOYD AND C. A. MAASKE

*From the Department of Physiology and Pharmacology
Loyola University School of Medicine, Chicago*

THE RESPIRATORY center, during each inspiration, normally receives impulses transmitted over the vagi from stretch receptors in the lungs. A state of central inhibition is thus built up, and eventually it cuts short the motor discharge. The actual mechanism of this effect is unknown. Both temporal and spatial summation of inhibitory impulses must be involved, for as the lungs are inflated there is a progressive recruitment in the number of active stretch receptors, together with an increase in the frequency of impulses from each unit (Adrian, 1933, Partridge, 1935). Inhibition might conceivably neutralize the driving force of inspiration, as it has been considered to inactivate quantitatively the central excitatory state (c e s) in spinal reflexes (Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932). Inspiration might then stop because the driving force is exhausted, the depletion resulting partly from neutralization, partly from expenditure in cellular activity. On the other hand, there may be no direct neutralization, inspiration ceasing because inhibition, when built up to a critical level, renders the center refractory to c e s. In the latter case the center, as it discharges, might still become increasingly susceptible to inhibition. It is possible that the resistance to inhibition might also be found to vary with alterations in the tension of CO_2 or of O_2.

It would be difficult, under natural conditions, to test these several hypotheses. The normal inflation reflex may, however, be eliminated by sectioning both vagi. Inspirations can then be regularly cut short by properly timed central stimulation of one vagus (Hillenbrand and Boyd, 1936). Under these conditions it is possible to observe the response of the center to groups of vagal volleys, the number and temporal distribution being controlled.

In a preliminary report Boyd and Hillenbrand (1937) described the effects of stimulating one vagus, during inspiration, for varying short periods of time. A single volley of impulses was never sufficient to inhibit. A definite "inhibitory threshold" could be found and expressed as the minimum number of volleys, or minimum time of stimulation required (frequency and shock voltage remaining constant). A threshold series of volleys would cut short inspiration promptly and completely, whereas a subthreshold series failed to reduce the amplitude at all. Graded reduction of inspiratory depth could be effected by varying the time at which stimulation began, but not by grading the number of inhibitory volleys. Apparently, therefore, inhibition does not quantitatively inactivate the driving force of inspiration. It

acts in an all-or-none manner, and the stopping of inspiration at any given point depends upon the intensity of inhibition existing at the moment.

The methods used in the present study make it possible for stimulation to be repeated at the same stage in successive inspirations. The stage at which it begins, the frequency, the voltage, and the duration of the period of stimulation, can all be controlled independently. We have made observations upon (a) certain effects appearing with a near-threshold series of volleys; (b) variations of the inhibitory threshold at different times during the course of inspiration; (c) the effects of increasing the respiratory dead space; and (d) the acceleration of rhythm which accompanies a reduction of amplitude.

It is recognized that when the vagus trunk is stimulated an inhibitory effect upon respiration may be due in part to afferent fibers not of pulmonary origin (e.g., from pressure receptors in the aorta). The electrodes were applied from 4 to 8 cm. below the origin of the superior laryngeal branch. A further complication is that fibers with an excitatory action upon the center may be stimulated simultaneously with the inhibitory group. Apparently the vagus trunk does contain fibers mediating pain sensations (Heinbecker and O'Leary, 1932); and the responses of lightly anesthetized animals to central stimulation of the vagus are somewhat variable. Tetanic stimulation for only 0.5 sec. may leave reflex after-effects of increased rate and amplitude persisting for several cycles. This is true particularly if strong stimuli are used. With adequate anesthesia, however, and with care to avoid unnecessarily strong stimuli, the effects of such brief stimulation are quite constant and are limited to a single cycle. The question of mixed excitatory and inhibitory effects will be again considered in section (d) below.

METHODS

Dogs were used, anesthetized by means of sodium barbital given intraperitoneally. The usual dose was 0.3 g. per kg., but in some instances this failed to induce a complete anesthesia and supplementary doses were given by slow intravenous injection. A tracheal cannula was inserted. Both vagi were sectioned in the low cervical region, and the central stump of one, ordinarily the left, used for stimulation. The electrodes, and the arrangement for recording respiratory changes of intrapleural pressure, were similar to those employed by Hillenbrand and Boyd (1936).

The stimulating circuit (Fig. 1) is based upon that described by Schmitt and Schmitt (1932). Brief induced shocks are set up by rhythmical discharges of a condenser (C_1 to C_3) through a coreless coil (T_1). We ordinarily used a single condenser of 0.02 μF., varying the frequency of discharge by adjusting the charging resistance R_1, and the stimulus strength by varying the shunt resistance R_6. Frequencies employed ranged from 80 to 140 per sec. The voltage necessarily was varied for different nerves, but was just sufficient in each instance to maintain, with continuous tetanization, a reflex apnea for a time equivalent to several cycles. Voltage and frequency are constant throughout each of the graphic records shown.

We modified the circuit of Schmitt and Schmitt by placing two RCA 885 tubes (A and B of Fig. 1) in parallel. The condenser discharges can be led through either tube by adjusting the grid potential of A, that of B being fixed. Stimulation takes place only when the discharge is through B, that tube being in series with the induction coil T_1.

With the switches K_5 and K_6 both open, the grid potential of A is low enough to divert all discharges through it, and the stimulating circuit is inactive. Closure of K_5 puts a fixed high potential on grid A, resulting in continuous tetanization until K_5 is opened again. Or, K_5 being left open, stimulation may be applied for any desired period, from a single shock
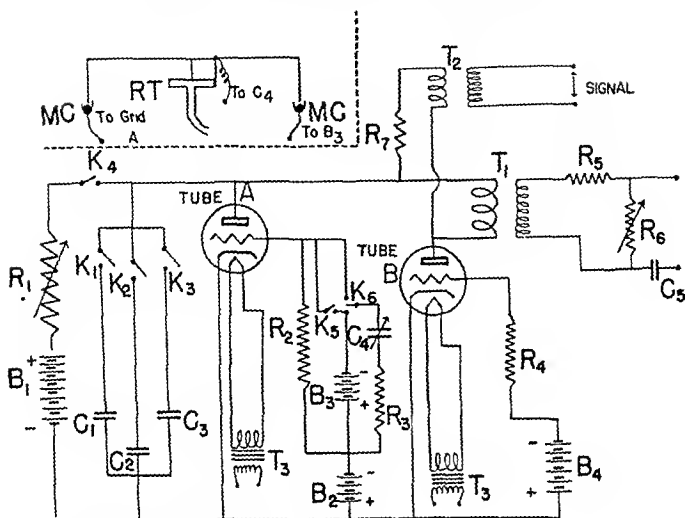
Fig 1 Details of stimulator A and B and device for automatic control
(RCA 885) B_1, B_2, B_3, B_4, batteries of 157 5, ____ ____ ____ ____ ance of 1 MΩ, variable in steps of 10,000 Ω R_1 ... ... 100,000, 20,000, 1000, and 500 Ω, respectively R_3, decade resistance variable from 1 to 10,000 Ω C_1, C_2, C_3, condensers of 0 1, 0 05 and 0 02 μF , usable singly or in combination by means of switches K_1 to K_3 C_4, a bank of condensers, capacity variable from 0 01 to 6 0 μF C_5, condenser of 0 1 μF to limit polarization at electrodes T_1, T_2, coreless induction coils, the first for stimulation, the second for controlling the grid in a signal circuit (not shown) T_3, T_3, a single transformer, operating from the 110 V A C circuit and supplying 2 5 V for heater filaments of tubes K_1, switch for continuous stimulation, K_6, double throw switch for charging and discharging C_4

Inset, upper left Device for automatic control of stimulator RT, tambour, MC, mercury cups Explanation in text

to about 1 5 sec of uniform tetanization, by varying the capacity of the condenser C_4 and using, to charge and discharge it, the double throw switch K_6 During the discharge of C_4 the grid potential of A is raised above that of B for a period of time which varies directly with the capacity of C_4 On the graphic records shown the stimulation time is indicated in terms of capacity, 1 μF corresponding to approximately 0 26 sec

Automatic control of the circuit by the animal's respiratory movements is made possible by substituting, for the switch K_6, the device shown in the inset of Fig 1 A tambour, connected to the intrapleural space, bears a long (30 cm), light lever, pivoted near the middle At each end of this lever a platinum wire projects toward a mercury cup below The cups are adjusted at such levels that one contact is maintained throughout the expiratory pause, charging the condenser C_4 The change of intrapleural pressure during inspiration first breaks this contact and later closes the second, discharging the condenser Vagal stimulation begins when the second contact is made It may be terminated by a reflex expiration which breaks the contact, but in any event it cannot continue beyond the limit of time set by the capacity of C_4

The "inspiratory" mercury cup is deep enough to avoid interference with the down-

ward movement of the lever. By raising or lowering this cup the discharging contact may be closed at an early or at a late stage of inspiration, as desired. The same device may be substituted for the single throw switch K_s, the "inspiratory" contact only being used. In that case stimulation, once started, continues until expiration breaks the contact. The coil T_2 (Fig. 1) is a duplicate of, and in parallel with, T_1. It has a resistance in series to limit diversion of current. It is used to control the grid of a third 885 tube, in an independent signal circuit. Some of our graphic records (Fig. 2, 3) were made before the automatic signal was added, and the periods of stimulation are not separately indicated.

RESULTS

a. *The effect of varying the number of vagal volleys in a series.* Responses to vagal stimulation at a frequency of 80 per sec., recurring at the same stage in successive inspirations, are shown in Fig. 2. The duration of the stimulation period is increased progressively from 0.31 sec. (1.2 μF.) to 0.52 sec. (2.0 μF.). The latter duration means that some 40 volleys are delivered, a number sufficient in this instance to cut short all inspirations regularly.
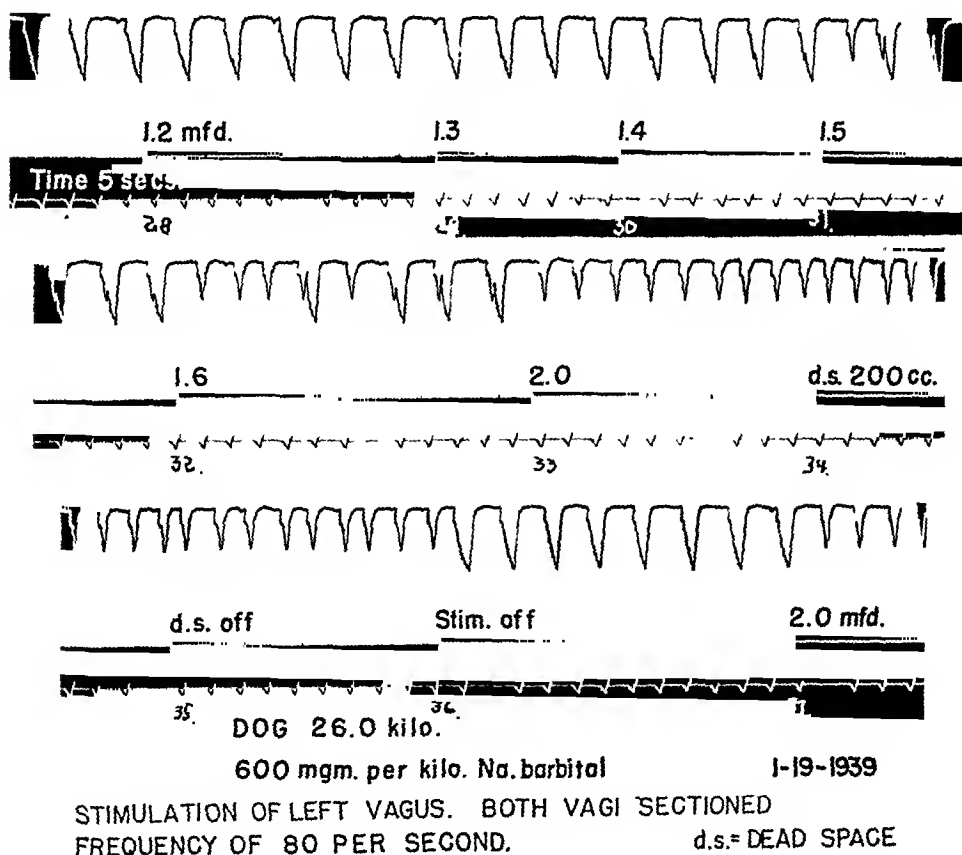
FIG. 2. Continuous record of respiration (intrapleural pressure, down stroke inspiration). Vagal stimulation, recurring at the same stage in successive inspirations, begins at first signal (top line) and continues to middle signal at bottom. Duration of stimulation periods progressively lengthened as indicated (1.0 μF. = 0.26 sec.). Showing (a) transient disturbance of inspiratory curve by a near-threshold series of volleys; (b) all or none effect on depth of inspiration; (c) inhibition still effective after dead space is increased.

Expiration goes to the normal level, and there is a definite, though abbreviated, expiratory pause. At 1.6 and 1.5 μF. there is a borderline effect. Some inspirations are cut short, others are merely interrupted by a rapid, incomplete expiratory movement after which the inspiration is resumed and goes on to full completion. The irregular responses in this near-threshold zone may perhaps depend upon accidental combinations of vagal impulses with other periodically recurring afferent influences, as from the carotid sinus.
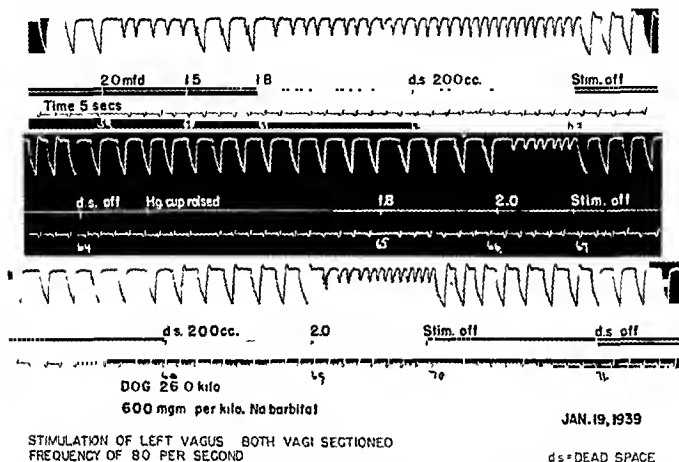
FIG. 3. Continuous record, same animal as in Fig. 2. First line, vagal stimulation for 0.39 sec. fails to cut short inspiration or to accelerate, but does both when the period is lengthened to 0.47 sec. The latter period of stimulation, however, is ineffective when applied earlier in inspiration (second line). Increase of dead space fails to raise the inhibitory threshold, although inspiration becomes slightly deeper (first and third lines). Note also that acceleration varies according to the stage at which inspiration is interrupted (compare first with second line).

With shorter periods of stimulation none of the inspirations are reduced in depth. The abortive expiratory movement becomes less marked, until, at 1.2 μF., it appears merely as a slight flattening of the inspiratory curve.

The abortive, partial expiration, caused by a near-threshold series of volleys, may mean either (i) brief reflex contraction of expiratory muscles, without an interruption of the inspiratory discharge, or (ii) momentary suspension of inspiratory activity followed by a "rebound" similar to that observed in spinal reflexes. By stimulating centrally the superior laryngeal nerve, it often is possible to keep an inspiration suspended at a mid-level for several secs. (Hillenbrand and Boyd, 1936). With the vagus, however, such an effect is in our experience always limited to a narrow range of

stimulation time,—usually somewhat narrower than in the example shown. It may be pointed out that the vagal "rebound" effect is obtained when the center is subjected to a near-threshold inhibition from which it is abruptly released, and presumably would not occur under natural conditions. The impulses from the stretch receptors do not cease sharply when inspiration stops, but die away gradually as the lungs are deflated (Adrian, 1933).

b. *The inhibitory threshold at different stages of inspiration.* The earlier vagal stimulation begins during inspiration, the longer is the stimulation period necessary to inhibit. Figure 3 shows the effect of raising the "inspiratory" contact. Before this change was made, stimulation for 0.47 sec. (1.8μF.) had been sufficient to cut short all inspirations regularly. Afterward

STIMULATION OF LEFT VAGUS. BOTH VAGI SECTIONED
FREQUENCY 100 PER SEC., 1000 OHMS   d.s.=DEAD SPACE
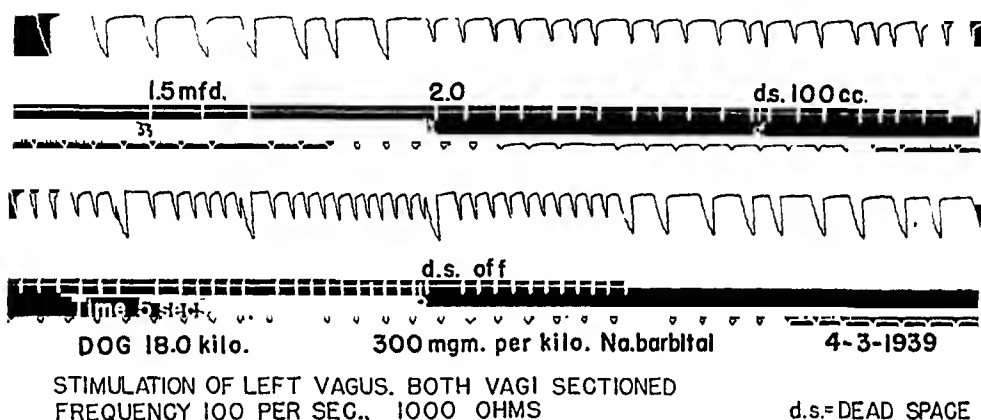
FIG. 4. Continuous record. With normal dead space vagal stimulation for 0.52 sec. cuts short all inspirations regularly. After increase of dead space an occasional inspiration breaks through (each stimulation period signalled).

the same stimulation period was found inadequate. Stimulation was then extended to 0.52 sec. (2.0 μF.) and inhibition again became effective. The lowering of threshold becomes quite marked during the final stages of inspiration. It is possible, of course, that the respiratory center is subjected during inspiration to inhibition from a higher center (Stella, 1938) or from receptors outside the lungs (Gesell, 1939); and that vagal stimulation becomes more effective, not because the center is inherently more susceptible, but because of the changing background of latent or subthreshold inhibition from these other sources. The behavior described, however, recalls the observation of Liddell and Sherrington (1925) that the crossed extensor reflex is more easily inhibtied in the latter part of its course than at the beginning.

c. *The effects of increased respiratory dead space.* With the tracheal cannula open directly to the outside air, we first determined the inhibitory threshold in the manner outlined in (a) above. A length of glass tubing (internal diameter 13 mm.), of 100 or 200 cc. capacity, was then attached

to the cannula. After a short time, respiratory stimulation was made evident by deeper and (usually) more rapid breathing. If the originally determined threshold stimulation was now applied to the vagus, recurring at the same stage of inspiration as before, the inspirations were still regularly cut short (Fig. 2, 3, 5). This procedure was followed with 11 dogs. Nine failed to show any elevation of the inhibitory threshold. With 2 animals (one instance is shown in Fig. 4) an occasional inspiration, after the dead space was increased, broke through the inhibition. On the other hand, the inhibitory threshold was sometimes actually lower after the dead space was increased (Fig. 5).

The failure of accumulating CO_2 to raise the inhibitory threshold was at first surprising, in view of the observations of Rice (1938). Rice applied

2.0 mld.   2.5   d.s. 100 cc.   2.5   2 2

2 0   d.s. off   2.0   2 2

Time 5 secs.   DOG 20.4 kilo.
300 mgm per kilo. Na. barbital

April 4, 1939

STIMULATION OF LEFT VAGUS   BOTH VAGI   SECTIONED
100 per sec,   700 ohms.   d.s.= DEAD SPACE
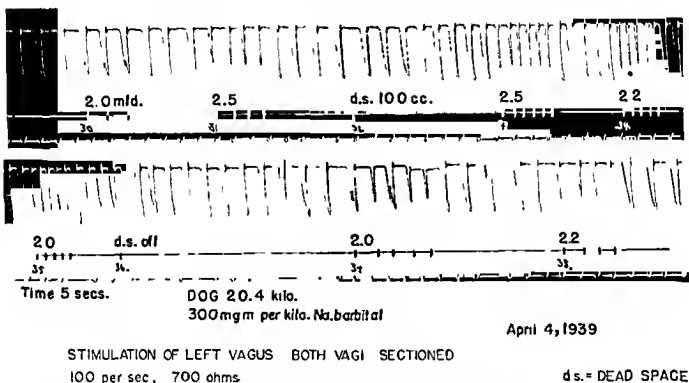
Fig. 5. Continuous record. Lowering of inhibitory threshold after dead space is in-increased. Vagal stimulation for 0.52 sec. cuts short all inspirations while the extra dead space is attached, fails to do so with normal dead space.

stimuli of uniform voltage and frequency to the central stump of one vagus (rabbit). The circuit was automatically closed at a fixed stage of inspiration and broken by expiration. With normal dead space, the inspirations were regularly inhibited at a relatively shallow level. With dead space increased the inspirations were still cut short, but not until they had been carried further toward completion.

Figure 3 of this paper shows a similar effect, though of small degree. After increase of the dead space the originally determined threshold stimulation of the vagus is still sufficient to inhibit, but the inspirations reach a slightly deeper level before they are cut short. The explanation apparently lies in the fact that under the influence of CO_2 the slope of the individual inspiration becomes steeper. This was noted by Rice, and is evident also on our records. When vagal stimulation begins at any given point on the

inspiratory curve, the final level reached will depend upon two factors. These are (i) the time required for temporal summation of the necessary number of inhibitory volleys, and (ii) the slope of the curve. When the latter becomes steeper there will be an increase of inspiratory depth even though the threshold of inhibition remains unchanged.

Rice's records (1938, p. 540) show an increase of depth exceeding any we have observed; but he used frequencies of stimulation somewhat lower than ours. The slower the frequency, the longer vagal stimulation must be continued in order to build up an effective inhibition; and the greater will be the difference in final level brought about by a given change in slope of the inspiratory curve.

d. *Concomitant changes in amplitude and rate.* Hillenbrand and Boyd (1936) noted that when an inspiration was inhibited by vagal stimulation, the ensuing expiratory pause was regularly shortened, provided care was used to avoid unnecessarily strong stimuli. This has been confirmed by Gesell, Steffensen and Brookhart (1937). The same acceleration of rhythm is manifest on the various graphs shown in this paper. It takes place too promptly to be attributed to changes in the chemical composition of the blood resulting from reduced ventilation. Moreover Adrian (1933) found a similar acceleration occurring when the inspiratory motor discharge was cut short by brief, properly timed inflation of the lungs (with vagi intact). Adrian suggested that the acceleration was an indirect consequence of inhibition, the center recovering more rapidly because it had not been allowed to discharge completely. The normal periodic activity of the stretch receptors, according to this view, has a dual effect; primarily it limits the amplitude of inspiration, but indirectly it keeps the rhythm accelerated.

Adrian's hypothesis seems to imply a reciprocal relation between the vagal effects upon amplitude and on rate. The earlier an inspiration is cut short the briefer should be the ensuing period of recovery. Hillenbrand and Boyd (1936) found that the acceleration does vary in degree according to the stage at which inspiration is interrupted. The effect upon rate becomes progressively less as the inspirations are allowed to go further toward completion. The same relationship is shown in Fig. 3 of this paper. The uppermost line shows a series of interrupted inspirations, with a marked acceleration of rhythm. The second line shows another series, cut short at an earlier stage and accompanied by a still greater acceleration.

Hammouda and Wilson (1935), however, hold that "augmentation of the rate of breathing cannot under any conditions be due to the stimulation of fibers carrying inhibitory impulses." They report that if the vagus be locally cooled to 8° C., the inhibitory fibers are selectively blocked. Continuous tetanization of the nerve then causes, not the usual reflex apnea, but an acceleration of breathing. Hammouda and Wilson believe that the afferent fibers responsible for this reversed effect are connected to pulmonary receptors; and that they are "augmentor" in function, tonically accelerating the respiratory rhythm.

The same authors, in a more recent paper, confirm the finding of Hillen-

brand and Boyd (1936) that breathing is accelerated by recurrent stimulation (limited to the inspiratory phase) of the uncooled vagus. This latter type of acceleration, they report, is lost when the vagus is cooled to 8°C. It therefore cannot be attributed to the "augmentor" fibers. They suggest (Hammouda and Wilson, 1939, pp. 522–523) that this particular type of acceleration is due to a third group of vagal afferent fibers, neither augmentor nor inhibitory, but having the same blocking temperature as the latter group.

We find, in agreement with Hammouda and Wilson, that cooling the vagus to about 8°C. abolishes simultaneously the inhibition of inspiration and the acceleration which accompanies it. But besides the identity of blocking temperature, the inhibitory and accelerator mechanisms have at least two other properties in common. First, the voltage threshold of the vagus is the same for both effects (stimulation being limited to inspiration). In preliminary tests at the beginning of each experiment we have used stimuli very weak at first but progressively strengthened. No acceleration ever has been observed from stimuli which were too weak to cut short inspiration. Acceleration regularly appears when the inspirations are inhibited, and not until then.

Second, if stimuli of adequate strength and frequency are applied, a period of stimulation which is too short to interrupt inspiration also fails to accelerate. If the period of stimulation is lengthened, acceleration appears as soon as the inspirations begin to be cut short, not earlier. This is shown in Fig. 2, 3, 4, 5. It is of course conceivable that two distinct groups of afferent fibers might have the same blocking temperature, the same voltage threshold, and might furthermore require, to produce their independent reflex effects, summation of exactly the same number of volleys; but such a coincidence seems rather improbable.

We therefore conclude that the reciprocal changes of respiratory amplitude and rate, demonstrated by Adrian (1933), by Hillenbrand and Boyd (1936), and in this paper, are so intimately related that they must be due to a common mechanism. The change of rhythm is evidently dependent in some manner upon the reduced inspiratory discharge. The opposing arguments advanced by Hammouda and Wilson (1939) will be considered in detail in a later publication.

SUMMARY

1. In dogs anesthetized with sodium barbital, and with both vagi sectioned, one vagus was stimulated centrally during inspiration. Adequate stimulation cuts short the current inspiration. This requires a certain threshold number of afferent volleys (voltage and frequency of stimuli being constant). The threshold number becomes less as inspiration advances.

2. Certain effects of a near-threshold series of volleys are described.

3. Graded reduction of inspiratory amplitude can be effected by varying the time at which the vagus is stimulated, but not by grading the number of inhibitory volleys. Vagal inhibition affects the respiratory center in an all or none manner.

4. Increase of the respiratory dead space does not raise the "inhibitory threshold" of the center. Inspiration becomes deeper, but this can be ac- accounted for by a more rapid inspiratory movement, and by the time required for inhibition to be built up to an effective level.

5. The cutting short of inspiration, by the procedure described, is ac- companied by an acceleration of rhythm. The amplitude and rate effects are related in the following ways:

(a) The voltage threshold of the vagus is the same for both.

(b) Both effects require temporal summation of a series of vagal volleys. The minimum number of volleys required to accelerate is also the threshold number for inhibition of inspiration.

(c) Both effects are simultaneously lost when a cold block of the vagus is gradually induced.

(d) The acceleration varies in degree according to the stage at which the inspirations are cut short.

6. It is concluded that the effects upon rate and amplitude are probably due to a common afferent mechanism, the acceleration being dependent upon the reduced motor discharge.

7. A stimulating circuit is described. It permits uniform tetanization for controllable short periods (0.01 to 1.5 sec.), frequency and stimulus strength being independently variable. The circuit can be automatically governed by respiratory changes of intrapleural pressure.

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CEREBELLAR ACTION POTENTIALS IN RESPONSE TO STIMULATION OF VARIOUS AFFERENT CONNECTIONS

ROBERT S. DOW

Laboratories of The Rockefeller Institute for Medical Research, New York

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INTRODUCTION

THE IMPORTANCE of the afferent fiber connections as a basis for functional localization in the cerebellum has been recognized since the work of Ingvar in 1918. From that time on extensive comparative anatomical investigations (Larsell, 1937) and ablation experiments (see Fulton and Dow, 1937, for recent summary) have supported a cerebellar division based on afferent connections. The importance of obtaining by still another method more specific information with respect to the afferent fibers is obvious. The present work is an attempt to confirm by an oscillographic method previously known afferent connections and to obtain additional information on the pontocerebellar and olivocerebellar connections. This method is advantageous for several reasons: (i) It gives direct evidence of the functional importance of the previously known afferent connections; (ii) it is not limited to myelinated fibers, as is the classical method of Marchi; and (iii) it is capable of easily establishing the presence of connections through one or more synapses.

HISTORICAL REVIEW

In 1912, Beck and Bikeles with the use of a sensitive galvanometer studied the effects on the cerebellum of single induction shocks applied to the sciatic nerve or to the nerves of the brachial plexus, and also the effects of thermal stimulation of the cerebral cortex. No pictures of the responses were given and only a list of the galvanometric deflections was presented in tabular form. When the peripheral nerves were stimulated, responses were found predominantly on the vermis of the cerebellum and not usually on the hemispheres. The nerves of the upper and the lower extremities were apparently equally capable of producing the effect, and as far as the responses were analyzed, the extent of the responsive area was identical following stimulation of the nerves of either the upper or the lower extremities. Thermal stimulation of the sensory motor cortex of the cerebral hemispheres gave a galvanometric deflection on both hemispheres, predominantly on the crossed one and occasionally on the vermis as well. There was no significant difference in the cerebellar response, whether the arm or the leg area was stimulated.

Camis in 1919 observed from the recordings of a string galvanometer that there was a change in the electrical activity of the cerebellum following mechanical or rotational stimulation of the vestibular apparatus. The change was not seen when the leads were placed on any part of the exposable cortex of the cat, but it could be observed when they were placed one in the fastigial nucleus and the other in the dentate nucleus, after removal of the overlying cortex. Price and Spiegel (1937) during rotation obtained an increase in the amplitude and frequency of the characteristic cerebellar activity in the most caudal part of the vermis

Attempts (Dow, 1938) to modify the "spontaneous" activity of the cerebellum by tetanic stimulation of peripheral nerves failed to produce any effects that could not be explained as secondary to a rise in blood pressure.
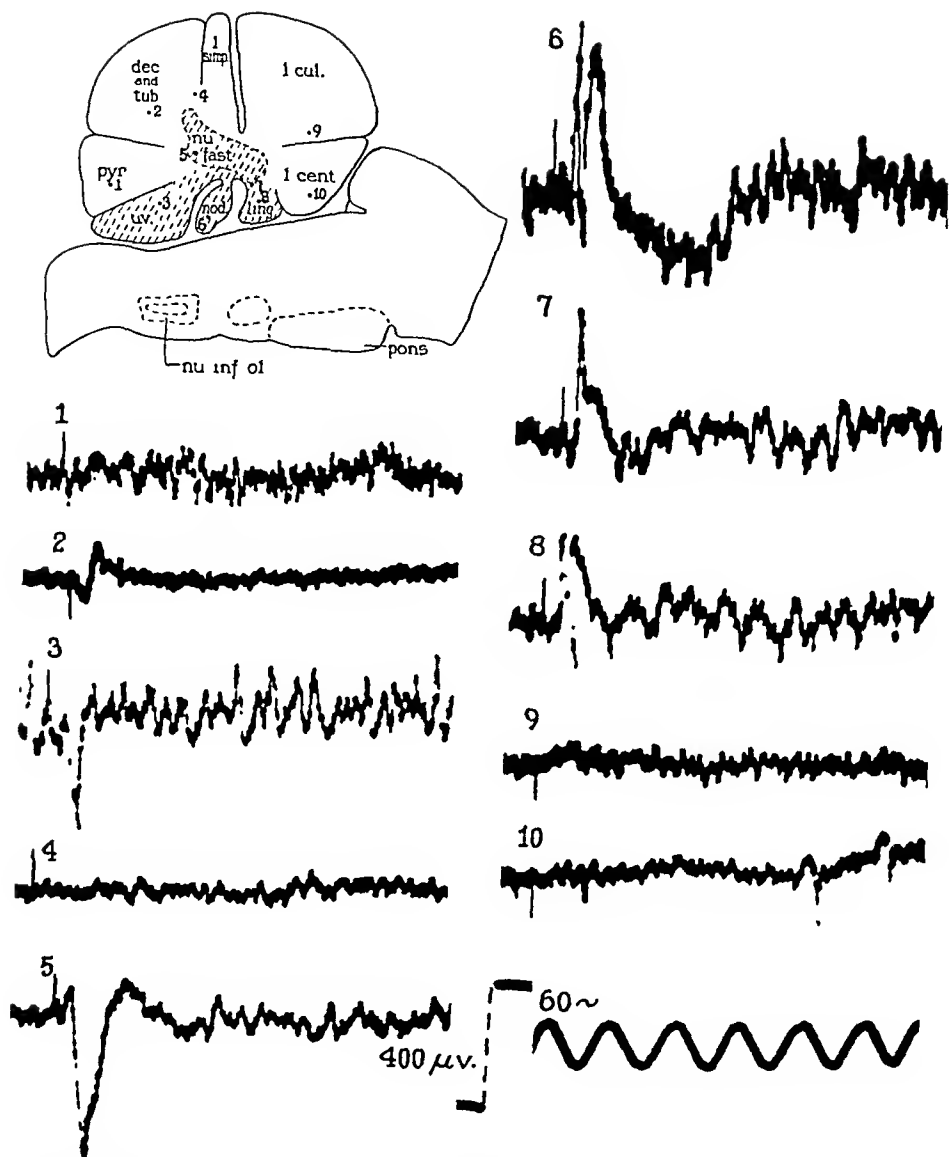
FIG. 1. Mid-sagittal diagram of cat's cerebellum. The shaded area is that in which responses to eighth nerve stimulation were found. The numbered points give the positions from which the oscillographic records were obtained in the experiment illustrated. The inactive points, 9 and 10, were active when the sciatic nerve was stimulated. Records taken with bipolar needle electrodes with bared tips, 1 mm. apart.

dec. and tub.,	declive and tuber vermis
l. cent.,	lobulus centralis
l. cul.,	culmen
l. simp.,	lobulus simplex]
ling.,	lingula
nod.,	nodulus
nu. fast.,	fastigial nucleus
nu. inf. ol.,	inferior olivary nuclei
pons,	pons
pyr.,	pyramis
uv.,	uvula

Forty decerebrated cats were used in the present investigation Stimulation of the nerves was effected by means of condenser discharges of short duration The stimulus was delivered through an audio transformer to reduce the shock artifact After transection, the nerves were placed in a glass stimulating electrode which was buried in the limb muscles The cord was stimulated directly by applying a small Lucite crescent shaped plate, in which two wires had been embedded, directly to the lateral surface of the cord In order to eliminate as much as possible the effects of this stimulation on parts of the cord other than the spino cerebellar tracts, the cord was frequently sectioned transversely below the site of stimulation and longitudinally for 1 5 to 2 cm at the level of the site of stimulation The eighth nerve was stimulated by exposing it in the internal auditory meatus and applying thin silver wire electrodes directly to the nerve trunk The pons and the inferior olivary nuclei were stimulated through two steel needles 1 mm apart and insulated except at their tips

A differential amplifier (Toennies, 1938) and a cathode ray oscillograph were employed For surface leading the ground was placed on the animal head holder, the active grid on the cerebellum, the inactive grid on the skull This "unipolar" leading gave good localization on the surface When a needle electrode was used to lead from points within the cerebellum, potentials of small amplitude were occasionally led from lobes that gave no surface response Movements of the needle electrode as great as several milli meters did not change the amplitude, sign or shape of these responses Bipolar leads in the same positions revealed no appreciable potential gradients Consequently the potentials recorded were believed to arise at relatively remote loci In order to differentiate locally produced potentials from those arising at a distance, all regions of the cerebellum were explored with bipolar leads consisting of two needles insulated except et their tips and separated by one mm

The deep parts of the cerebellum were explored by systematically moving the bipolar electrodes to different points in a particular vertical plane by means of a mechanical manipulator, the movements of which could be measured accurately The positions of the electrodes were identified by the distance from an electrolytic lesion made at some point in the path of the needles and located at the end of the experiment in thin sections cut from the brain after it had been fixed in formalin After some experience it was found possi ble to use physiological landmarks as aids in locating the electrodes in the course of an experiment

EXPERIMENTAL RESULTS

Stimulation of the eighth nerve Responses to stimulation of the eighth nerve were found in the flocculonodular lobe, the uvula, lingula, and fastigial nucleus (Fig 1) The potentials were obtained by using bipolar needle electrodes and systematically exploring all the lobes The latency—the interval between the shock artifact and the beginning of the first potential clearly separate from any "spontaneous" activity—in the midline cerebellar structure was 4 5 msec In one experiment with the lead electrodes at the base of the nodulus, in addition to the response beginning at 4 5 msec there was a spike-like potential having a total duration of only 1 8 msec This potential began 0 6 msec after the shock artifact and probably was produced by the vestibulocerebellar nerve fibers It is known from degeneration experiments that in the cat primary myelinated vestibular fibers enter the cerebellar cortex at this point (Ingvar, 1918, Dow, 1936) There were true cerebellar action potentials in the homolateral flocculus after a latency of 3 msec It is impossible to know whether or not earlier potentials, such as the spike recorded at the base of the nodulus, were present, because in some experiments (Fig 2) the shock artifact when leading from the homo lateral flocculus interfered with the record for as long as 2 5 msec A response

was seen in the contralateral flocculus in the one experiment in which the contralateral lobes were explored.

These lobes and nuclei were the only ones from which potentials could be obtained when bipolar needles were used. Occasionally potentials of low amplitude resulted from other lobes with unipolar leads; but their size and
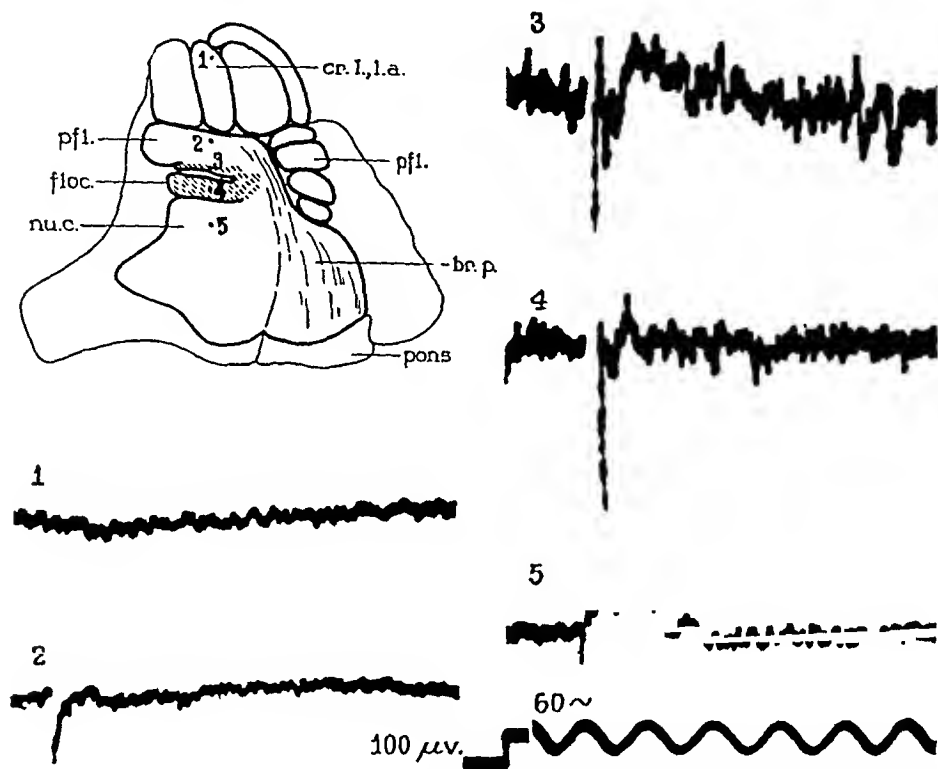
FIG. 2. Para-sagittal diagram of the cat's cerebellum with points indicating the position of the leads in an experiment in which the eighth nerve was stimulated. Responses were obtained in the flocculus, but not in the lobulus ansiformis and paraflocculus. Point 5, in dorsal cochlear nucleus. Records taken with bipolar needle electrodes with bared tips, 1 mm. apart.

br. p.,	brachium pontis
cr. I., l. a.,	crus I, lobulus ansiformis
floc.,	flocculus
nu. c.,	dorsal cochlear nucleus
pfl.,	paraflocculus

the failure to change in form with movement of the needle indicated that they were not produced in the region of the leads.

The facts reported above would not in themselves differentiate between responses to stimulation of the vestibular division of the eighth nerve and of the auditory division. That the responses are caused by stimulation of the auditory division is unlikely on anatomical grounds, and from the work of Camis (1919) and of Price and Spiegel (1937) we have evidence that physio-

logical stimulation of the vestibular apparatus can result in an alteration of the electrocerebellogram. In the course of the present work there was no effect from sound stimulation in any part of the cerebellum, including the lobes giving the most marked responses to vestibular impulses, although

FIG 3 Mid-sagittal diagram of cat's cerebellum The shaded area shows lobes responding to stimulation of spinal nerves Numbers of points and records correspond, taken from a single illustrative experiment Note that the principal responses occur throughout the anterior lobe Record 2 from pyramis shows a slight effect, considerably less than usual Records taken with bipolar needle electrodes with bared tips, 1 mm apart Normal activity of some of the silent areas was controlled by stimulation of the pons in this experiment Vestibular stimulation used as control of activity of the uvula and nodulus in other experiments

responses in the acoustic tracts and nuclei to both sound and electrical stimuli were regularly obtained.

The presence of potentials arising locally in the flocculonodular lobe, the lingula, uvula, and the fastigial nucleus, and their absence in other lobes and nuclei, are in accord with the anatomical findings of Ingvar (1918) and of Dow (1936) on the distribution of primary vestibular cerebellar fibers in the cat. If the distribution is the same in the cat as in the rat, it is also in

accord with the location of the terminations of the secondary vestibular fibers (Dow, 1936).

Stimulation of the spinal nerves or spinal cord. The sciatic nerve was chosen for the majority of the 16 experiments of this group. When the nerves of the upper extremity were stimulated, the median and ulnar nerves were used. The cord was stimulated directly in the mid-dorsal region. The entire anterior lobe, the lobulus simplex, pyramis, and occasionally the lobulus paramedianus responded to stimulation of the spinal cord or its nerves. The declive and tuber vermis, the lobulus ansiformis, paraflocculus, uvula, and flocculonodular lobe gave no potentials in response to spinal stimulation (Fig. 3). The effect was bilateral, but more marked on the homolateral side. When recorded from surface electrodes, the potential was frequently complex, but the predominant potential deflection was surface positive. The beginning of the potential resulting from stimulation of the sciatic nerve was usually seen after a latency of from 8 to 13 msec., depending upon the experiment and the part of the cerebellum from which the potentials were led. In a few experiments high amplification revealed an earlier surface positive wave, in one instance after only 4.5 msec. Because this early wave was most frequently seen when the lead electrode was near the peduncles and because of its short latency, it may be a record of the potentials of the incoming spinocerebellar fibers. The latency following stimulation of the nerves of the brachial plexus varied from 3.5 to 5 msec., depending upon the experiment and the part of the cerebellum from which potentials were led. When the cord was prepared so that the spinocerebellar fibers were stimulated directly in the mid-thoracic region, the first potential, always surface positive, was seen after a latency of only 1 msec.

The lobes responding were the same, regardless of the site of stimulation. Indeed, in the experiment shown in Fig. 4 one could compare point for point throughout the entire exposable cortex the effect of stimulation of the nerves of the upper extremity with that of the lower. Not only were there potentials in the same lobes, but frequently even the form of the potential was remarkably similar. This was true even when the animal was under ether anesthesia deep enough to eliminate any possible reflex effects from one extremity to the other. The finding suggests that the same elements may be activated by stimuli arising in either the upper or the lower extremity. The distribution and complexity of the response at a given point were unchanged when the reflexes were abolished by curare. If the animal was anesthetized with dial and not decerebrated, the distribution of the spinal responses was the same, in so far as the exposure allowed an exploration of the surface of the cerebellum. No physiological stimulation has been attempted in the present experiments. Stimulation of the saphenous nerve, which is cutaneous in distribution, resulted in a cerebellar potential, which was still present after all reflexes had been abolished by a large dose of curare.

Responses in the "spinal parts" of the cerebellum may be obtained from stimulation in the dorsal reticular formation (Fig. 7). Whether they are

the result of the stimulation of nuclei situated there, or of fibers passing through the brain stem on their way to the cerebellum, cannot be determined from the data at hand.

Stimulation of the pons. Responses from the pons were obtained in the

Fig 4 Schematic diagram of folial pattern of cat's cerebellum The shaded area shows the lobes responding to stimulation of nerves of right upper and lower extremities The degree of shading is a rough index of the amplitude of the responses and shows diagrammatically the tendency for the greatest response on homolateral side Records from a single experiment allow a comparison, point for point, of the effect of stimulation of nerves of upper and lower extremity Note the correspondence in area responding and striking similarity of potential (records 3 and 4) Note apparent increase in "spontaneous" activity on contralateral side after nerve stimulation, record 1. "Unipolar" cotton wick electrode used

cr II, l a ,	crus II, lobulus ansiformis
fis p ,	fissura prima
fis po lat ,	fissura postero-lateralis (Larsell)
fis sec ,	fissura secunda
fis ppd ,	fissura prepyramidalis
lo ant ,	anterior lobe
l. p med ,	lobulus paramedianus

lobulus ansiformis, paraflocculus, lobulus paramedianus, and the declive and tuber vermis (Fig. 5). At times the culmen lobulus simplex and pyramis also gave responses of a somewhat lower amplitude. The response usually consisted of a surface positive potential of spike-like proportions which appeared out of the shock artifact and was followed by a much longer lasting

potential, usually surface negative. The slower potential was occasionally surface positive, especially when a record was made from lobes caudal to the fissura prima. When no potentials were obtained from the surface, bipolar needles thrust below the surface likewise revealed no potentials
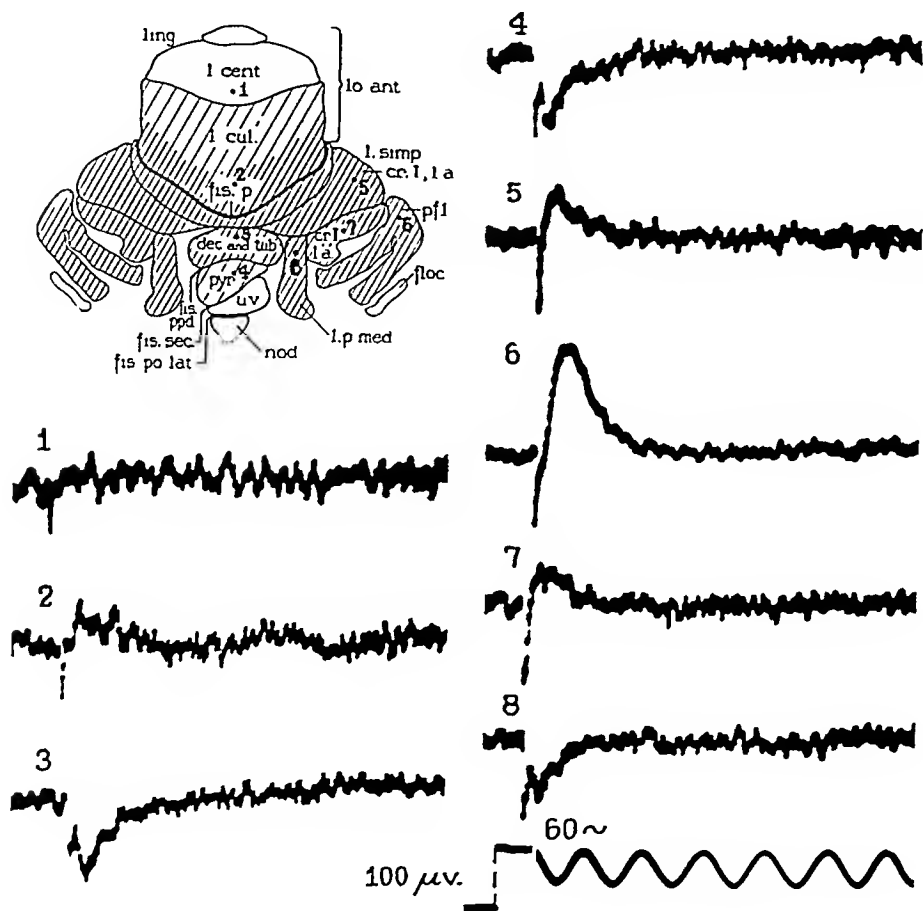
FIG. 5. Schematic diagram of folial pattern of cat's cerebellum. The shaded area gives the lobes responding to pontine stimulation, and the intensity of the shading furnishes a rough index of the relative amplitudes of the responses ordinarily encountered. Numbers of points and records correspond; taken from single illustrative experiment. "Unipolar" cotton wick electrode used. Note that almost the whole of the exposable cortex in the cat shows a response to stimulation of the pons.

(Fig. 6); but unipolar needles revealed low potentials that, as in the experiments in which the vestibular nerve was stimulated, probably represented action at a distance.

As yet no conclusions have been reached regarding the differential distribution of fibers from various parts of the pons, for example, the pre- and post-trigeminal parts of the pons which, as indicated by comparative

evidence (Abbie, 1934), may have a different distribution. The experiment shown in Fig. 6 suggests that the pontine projection to the anterior lobe arises only in the most posterior parts of the pons. A stimulus more localized than has been desirable in these experiments may bring some evidence to bear on this point. Activation of the pontocerebellar projection synaptically, by cerebral cortical stimulation, has not been attempted; in view of the
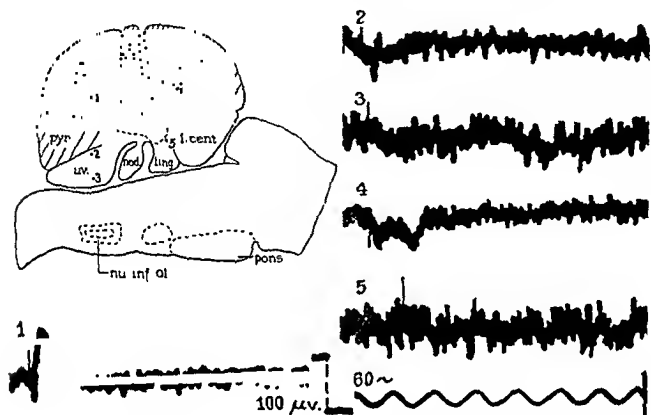
FIG. 6. Mid-sagittal diagram of cat's cerebellum. The shaded area shows the lobes responding to stimulation of the pons. The degree of shading gives a rough index of the relative amplitudes of the usual responses. Numbers of points and records correspond; taken from single illustrative experiment. Point 4 gave no response when the stimulating electrode was in the rostral part of the pons, but gave the response seen in record 4 when the caudal part of pons was stimulated.

effects of thermal stimulation reported by Beck and Bikeles (1912) it should yield interesting results.

Stimulation of the inferior olivary nuclei. Stimulation of this region gives responses in all parts of the cerebellum including the flocculus. The responses of greatest amplitude were found in the contralateral hemisphere, but homolateral responses were also seen (Fig. 7).

The responses, usually surface negative, were often preceded by a surface positive potential and were seen after a latency of 5 to 5.5 msec., depending upon the experiment. Occasionally the relation of the surface lead electrode to a fissure had great influence upon the sign of the potential. With identical lead positions there occurred a characteristic difference in the sign of the responses, depending upon whether the dorsal reticular formation, or the inferior olive, was stimulated. Movement of the stimulat-

ing electrodes by only 3 mm. was sufficient to change not alone the sign of the response, but also the extent of the cortex responding and the recovery characteristics.

The olive response was exceedingly sensitive to conditioning by previous activity. The response to the second of two maximal shocks was often com-

FIG. 7. Mid-sagittal section of cat's cerebellum showing position of stimuli, S_1 and S_2, and a folial pattern showing the position of the leads L_1 and L_2. a, b, c: Stimulus S_1 in dorsal reticular formation and lead L_1 on ventral part of culmen. Responses surface positive. a, Conditioning alone; b, testing alone; c, both. Note that the inhibition of the second response is very slight. d, Stimulus S_1 and lead L_2 on the lobulus ansiformis. Note absence of response. e, f, g: Stimulus S_2 in inferior olivary nuclei, lead L_1. Responses now surface negative. e, Conditioning alone; f, testing alone; g, both. Note marked conditioning. h, i, j: Stimulus S_2, lead L_2. Almost complete inhibition by a conditioning action 65 msec. before the test. "Unipolar" cotton wick electrode used for the leads.

pletely suppressed when the second shock came 70 msec. after the first; and the periods of subnormality lasted as long as 5 sec. Leads from the olive showed that the conditioning was in the olive itself and not in the cerebellum. It would appear that a stimulus in the region of the olive or just rostral to it excites synaptically the whole of the homolateral nucleus. The interconnections within the olive are not well-known. Occasionally there was a response in the cerebellar cortex that was not completely inhibited by a previous shock, which suggested that the olivocerebellar fibers, or their cells of origin, were stimulated directly. These less readily inhibited

responses were not widely distributed over the cerebellum, as were the more readily inhibited ones. It is possible that by taking advantage of this method of separation of direct and indirect excitation of the olivocerebellar connections, and by using more localized stimulation, details concerning the point to point relationship between parts of the olivary nuclei and specific lobes of the cerebellum may be worked out oscillographically.

The difference in the responses led from the same point when two totally different afferent systems are stimulated, as for instance the inferior olivary nucleus and the spinocerebellar tract (Fig. 7), suggest that different elements of the cerebellar cortex, or different pathways in the cortex, are involved. A possible anatomical basis for the difference lies in the presence of two systems of afferent fiber connections to the cerebellar cortex, namely the mossy fibers with their connection to the granular cells and thence to the Purkinje cells, and the equally important climbing fibers that have direct synaptic connections with the Purkinje cells.

Although the present data do not identify the elements within the cerebellar cortex responsible for the potentials, evidence does seem to point to the cerebellar neurons as the chief source of the potentials, rather than to the afferent axons and their endings. The evidence rests upon the fact that the synaptic region of neurons is much more dependent upon readily available oxygen than are nerve fibers. The cerebellum was rendered ischemic by clamping the carotid arteries in a preparation in which the vertebral arteries had previously been ligated. During the clamping, artificial respiration was kept up in order to prevent a falling off of the oxygen supply of the spinal cord because of depression of the respiratory center. Twenty seconds after the clamps were applied, the "spontaneous" activity began to be affected and at the same time there was a decrease in the response to sciatic stimulation. At the end of 30 sec. when the spontaneous activity had disappeared, the response was further reduced, but it did not disappear altogether until 45 min. had elapsed.

The response in the cerebellum to stimulation of the cerebellar tracts in the spinal cord was depressed in a similar manner by asphyxia or ischemia. In this instance, however, a potential small in comparison with the original one could still be lead from the anterior lobe after 15 min. The potential undoubtedly originated in tract fibers, and it served as a contrast to the neuron potentials which were depressed in a period measured in seconds.

The arrival of a single afferent volley and the response of the cerebellar cortical neurons have remarkably little effect upon the background or the so-called "spontaneous" activity which is characteristic of the cerebellum and which is known to vary in amplitude and frequency as the effect of the cerebellum upon muscular tonus varies (Dow, 1938). However, in a few experiments in which for some reason the spontaneous activity was decreased, the arrival of an afferent volley set off a short chain of rhythmic waves at the usual frequency of 150 to 250 per sec. If was a regular observation following stimulation of a spinal nerve that the response on the contra-

lateral side of the anterior lobe, although not as large as that on the homo-lateral side, was associated with a definite increase in the amplitude and frequency of the background activity (Fig. 4 1). This effect might be caused by fractionation of the response on the edge of the responding field, or by the activation of laterally conducting elements within the cerebellar cortex. In no instance was there any suppression of the activity following a single afferent stimulation. These observations, as far as they go, are in agreement with those of Price and Spiegel (1937) who found an increase in the amplitude and frequency of the cerebellar potentials as recorded from the uvula during rotation of the animal.

Conclusions

1. Single shock electrical stimulation of afferent connections to the cerebellum results in a response of the neurons of the cortex in the lobes with which the respective afferent system has connections.

2. Stimulation of the eighth nerve in the unanesthetized decerebrated cat results in an electrical response in the cerebellum, limited to the flocculo-nodular lobe, the lingula, uvula, and the fastigial nucleus.

3. Stimulation of the spinal nerves, or of the spinocerebellar tract in the mid-thoracic cord, produces cerebellar action potentials limited to the anterior lobe, the lobulus simplex, pyramis, and occasionally the lobulus paramedianus. No difference in location of the response depending upon the site of stimulation was seen, except for a response of greater amplitude on the homolateral side.

4. Stimulation of the pons sets up cerebellar action potentials limited to the middle lobe of the vermis (Ingvar), the lobulus ansiformis, lobulus paramedianus, paraflocculus, and pyramis. Occasionally potentials are found also in the dorsal part of the culmen.

5. A stimulus in the region of the inferior olivary nuclei excites synaptically the olivocerebellar connections and produces cerebellar action potentials in all the lobes of the cerebellum. The responses of greatest amplitude are found when leading from the contralateral lobulus ansiformis.

6. In similar afferent systems, such as the nerves of the upper and the lower extremities, the responses led from an identical point may be strikingly similar. With dissimilar afferent systems, such as the inferior olive and the spinal system, potentials of different sign and shape may be led from the same point. The question is raised whether this dissimilarity of potential depending upon the source of the afferent stimulation may not be attributable to a difference in the anatomical connections within the cerebellar cortex.

7. It is thought that these action potentials are due to activity of the neurons of the cerebellar cortex and that they are not potentials of the axons or endings of the afferent fibers. In most instances the activity resulting from single shocks changes to a remarkably small degree the background activity which is characteristic of the cerebellar cortex. Under certain con-

ditions, however, this activity may be initiated or increased by a response to an afferent stimulation. In no instance has a decrease been observed.

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CHANGES IN RETINAL EXCITABILITY DUE TO POLARIZATION AND SOME OBSERVATIONS ON THE RELATION BETWEEN THE PROCESSES IN RETINA AND NERVE*

RAGNAR GRANIT AND TOIVO HELME

From the Physiology Institute, Helsingfors University

INTRODUCTION

THE RETINA would seem to be a useful preparation for studying the effect of a polarizing current on the excitability of sensory neurones relaying impulses to other neurones. We have undertaken such experiments and, although their interpretation is difficult, the results are clear cut and at least give some information about the relation between the processes in the retina and the optic nerve. Because of their significance for the problem under discussion some observations on the effect of antidromic impulses into the
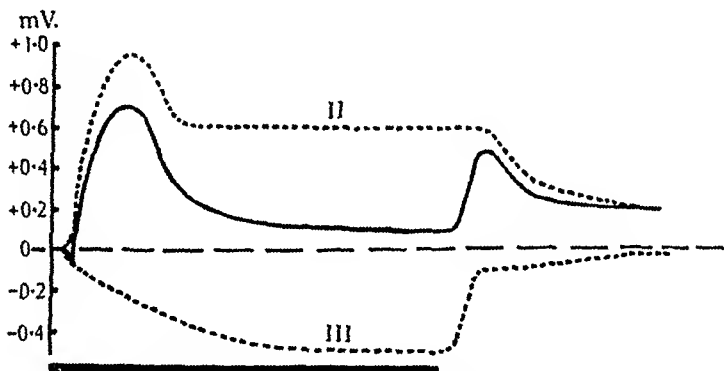
FIG. 1. Analysis of light-adapted frog's electroretinogram according to Granit and Riddell, 1934. See text.

optic nerve and on the latent period of the retinal and nerve processes have been added.

For the purpose of this paper we can neglect the slow secondary rise of the retinal electrical response, known as P I. We are here chiefly interested in the two remaining potentials of opposite sign, both of which are directly connected with the states of excitation and inhibition set up by light. Actually this restriction only means that we are dealing with the light-adapted frog's eye since the slow component P I requires dark adaptation.

The electroretinogram of the light-adapted frog's eye is shown in the diagram of Fig. 1, which also illustrates its analysis into components. The off-effect is solved as an interference phenomenon due to the negative P III returning to the base line faster than the positive P II (Granit and Riddell,

1934) Later work by Granit and Therman (1937) has suggested the necessity of introducing a renewed rise of P II at cessation of illumination For the cat's eye, where the off-effect is merely a retardation of the fall towards the base line at "off," the interference-theory still would seem to be valid (Granit, 1933) Further details may be found in recent publications by Hartline (1937, 1938), Granit (1938), and Granit and Therman (1937) It may further be added that the diagram of Fig 1 refers to the standard leads cornea, lens, or vitreous body⟷back of the bulb Neither P II nor P III can be explained as the sum total of rapid spikes of impulses (see, e g Granit, 1938, Therman, 1938)

METHODS

The amplifying and recording technique used in several earlier contributions to similar problems has been employed A double cathode ray one directly coupled push pull ampli-
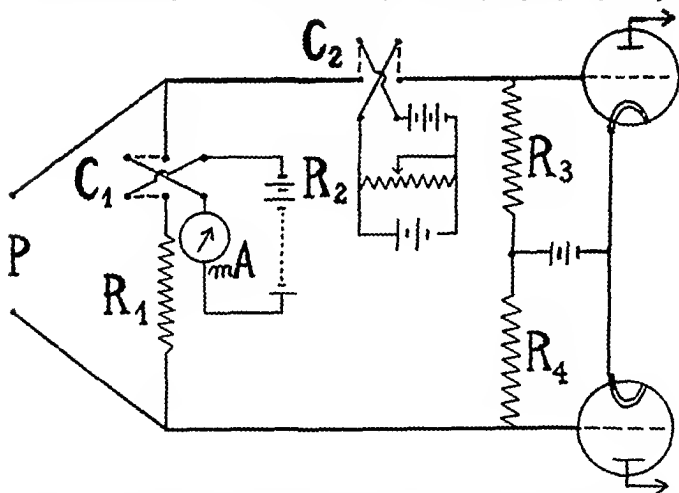
FIG 2 Diagram of polarizing and compensating circuit in the input of the amplifier P preparation (frog's retina) between electrodes used for leading off and for polarizing R_1 0 5 MΩ, R_2 1000 Ω potentiometer, $R_3 = R_4$ 0 5 MΩ, mA milliammeter, C_1 and C_2 commutators

fier, and two balanced condenser coupled amplifiers have been available for leading off from silver silver chloride electrodes on the excised opened frog's eye (standard lead) and its optic nerve The pair of balanced amplifiers was used for simultaneous records from retina and nerve, the directly coupled instrument when it was necessary to obtain an undistorted picture of the electroretinogram

Polarization across the standard retinal leads offered no problem when records were taken from the optic nerve But with the eye itself between the leading off electrodes steps had to be taken to avoid blocking of the amplifier by the polarizing current In some ex

periments this was done by putting a pair of $4\mu F$ condensers in series with the input, but a better method enabling use of the properties of the directly coupled amplifier was found to be the one illustrated in Fig. 2. Also in this case silver-silver-chloride electrodes were used though some experiments also were carried out with calomel half-cell electrodes.

In Fig. 2, illustrating the input of the push-pull amplifier, the polarizing current containing the commutator C_1 traverses the retina over the large resistance R_1 smoothing out changes in the resistance of the preparation (about 10,000 Ω). The circuit compensating for the drop of potential across P contains batteries, the commutator C_2 and potentiometer R_2 for balancing out the amplifier. The two commutators C_1 and C_2 are switched over simultaneously. As the effect of polarization on the retinal response is reasonably constant for several minutes, there is ample time for adjusting the sensitivity of the amplifier and waiting for the initial drift caused by the polarizing current to cease before the eye is illuminated.

RESULTS

1. *Leading off from the nerve.* The input of the amplifier was connected to electrodes on the whole nerve and the excised opened eye stimulated by light during the passage of a galvanic current across the standard leads. For reasons to be explained below it is important to note that the opened bulb should not be allowed to become dry. "Inside cathode," "inside anode" means that the cotton wick touching the fluid inside the bulb is cathode and anode respectively.

With this arrangement easily repeatable results were possible. For galvanic currents between 0.3–1.0 mA the optic nerve response to the test light was greatly enhanced by "inside cathode" and greatly diminished by "inside anode." This is shown in Fig. 3. With the whole nerve and relatively low sensitivity the effects at "on" and "off" completely dominate the picture of the optic nerve response of the light-adapted eye. The range in mA within which

FIG. 3. Effect of polarization on the discharge through the optic nerve. Illumination for 10 sec. marked on film above time in 1/5 sec. Relatively low sensitivity in order to obtain the main deflexions at onset and cessation of illumination. A, normal on- and off-effects; B, "inside anode," polarizing current 0.4 mA; C, "inside cathode," polarizing current 0.4 mA. Amplifier in this case thrown out of its working range by the large deflexions at "on" and "off."

these effects were obtained varied from eye to eye. Weak currents had little effect and stronger ones introduced various complications. On increasing current strength above the limits mentioned the typical effect was a diminution of the responses to both "inside cathode" and "anode," relatively greater for the latter. With "inside anode" and gradually increasing current strength the impulses could be almost completely blocked before the response to "inside cathode" had diminished by more than 50 per cent.

Differences in the relative effect of the polarizing current on the outburst of impulses at onset and removal of the stimulating light were fairly regularly seen. If, before polarization, the off-effects are large they increased or diminished in the same proportion as the on-outburst during the passage of the galvanic current. But if, to begin with, the off-effects were small, then they were more sensitive to the effect of polarization than the initial reaction to light. "Inside cathode" often made the off-effects relatively larger, and "inside anode" relatively smaller than the reactions to the onset of illumination. With very strong polarizing currents the off-effects were the last to disappear with "inside cathode" and the first to go with "inside anode."

2. *Nature of anomalous results.* Mixed atypical effects as well as reversal of the normal findings described above were sometimes obtained. But it proved possible to locate the source of error. The decisive factor turned out to be the amount of fluid or vitreous body at the inside electrode. Localization of this electrode to the retina immediately lead to unpredictable complex effects. These sometimes arose spontaneously when the cotton wick inside the bulb had drained away its content of fluid. In both cases normal results were immediately restored by the simple expedient of filling the bulb with Ringer solution.

From the point of view of the distribution of current between the poles of the galvanic source its concentration to a differential electrode touching the retina would seem to be a very complex affair. Our intention of placing the retina as a whole between the poles of a current distributed as uniformly as possible would seem to be realized only by the experiments in which the bulb was filled with fluid or the inside electrode was touching the lens floating in the middle of the opened eye.

3. *Effect of polarization on the retinal electrical response.* In order to find out whether polarization affected retina or nerve it was necessary to record the retinal electrical response itself. The leads to the amplifier were connected to the electrodes used for polarization and the compensatory device described in the section of methods was put in. Alternatively, in a few experiments, the input leads of the amplifier were blocked with condensers. Both methods gave identical results. "Inside cathode" was found to increase the retinal electrical response and "inside anode" to diminish it. In all essentials these facts were a replica of those obtained with the nerve. This is shown in Fig. 4. Hence polarization seems to affect the retina directly. The results obtained with the nerve must be due to changes in the retina.

The first effect of the polarizing current naturally was a drift of base line of the recording instrument. But as the effects of "inside cathode" and "inside anode" persisted for several minutes with exposure at intervals of 30 sec. it was possible to study them after compensation of this drift.

4. *Effect of polarization on the isolated negative component P III of the retinal response.* It is known (Granit and Therman, 1937; Therman, 1938) that a drop of KCl-solution applied on to the retina quickly removes the positive component P II of the electrical response to light and leaves a pure negative deflexion, which has the latency of the initial *a*-wave and returns to the base line in a way imitating the properties of the off-effect of the
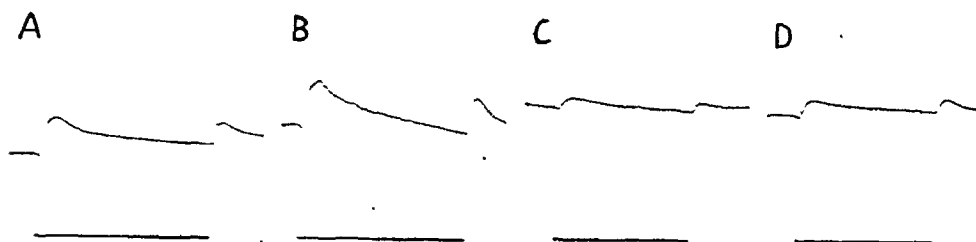
Fɪɢ. 4. Effect of polarizing on the retinal electrical response. A, normal electroretino-gram; B, polarization (0.4 mA) with "inside cathode"; C, polarization (0.4) with "inside anode"; D, control. Note that the retinal electrical response has diminished from A to D. The characteristic effects of polarizing are nevertheless easily distinguished.

complete retinal action potential. This deflexion is identical with the negative component P III (Granit and Riddell, 1934). Before any change can be seen in the retinal action potential after potassium, the discharge in the optic nerve is blocked by the poison.

The eye gradually dies under the influence of a dose of KCl capable of removing selectively the positive component P II but the gradual disappearance of the negative P III is slow enough to enable a sufficient number of "anodes" and "cathodes" with intervening "controls" to be superimposed upon the slow decline of the electrical response to light. Figure 5 shows that the negative component P III reacts to polarization in precisely the same manner as the discharge in the nerve and the complete electrical response of positive sign. "Inside cathode" enhances, and "inside anode" depresses the negative component P III.

5. *Conclusion.* Let us consider what these facts mean in terms of the components of the retinal action potential. At the onset of illumination P II and P III are trying to deflect the galvanometer in opposite directions. If the two components were of equal potential and equally enhanced or diminished by polarization there would be complete cancellation of these effects at "on," whereas at "off" they would add. Thus, in general, "inside cathode" should favour the off-effects, and if, as we have seen, the *b*-wave also is larger it can only mean that the positive component P II has been

increased by "inside cathode" more than the negative component P III.

The increased retinal off-effect with "inside cathode" cannot be a simple interference phenomenon, due to P III returning from a lower level and without physiological significance as it is associated with an equivalent increase of the total discharge in the optic nerve.

6. *Antidromic impulses.* Already in 1932 one of us (R.G.) together with Dr. J. C. Eccles tried firing antidromically into the optic nerve of the decerebrate cat without being able to demonstrate any effect on the electro-

A   B   C
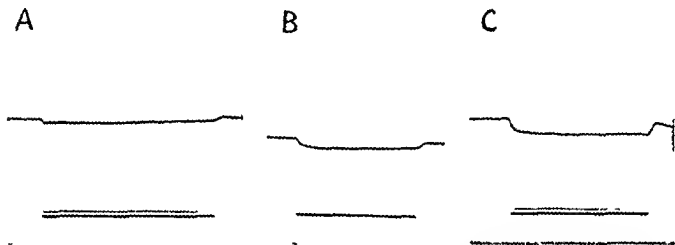
FIG. 5. Effect of polarizing on a retinal electrical response made negative (P III) by a drop of KCl into the eye. A, "inside anode" (0.4 mA); B, polarizing current removed; C, polarization with "inside cathode."

retinogram. These experiments were now repeated with the more accessible nerve of the frog's eye. The stimuli came from a neon stimulator, the cathode of which was lying towards the retinal end of the nerve. Widely different rates of stimulation were employed and the eye was illuminated for a few seconds at regular intervals leaving, as one would expect, the antidromic impulses a chance of being timed in every possible way relative to the onset of the retinal action potential. No effect whatsoever, excluding the rapid shock artefacts, was noticed. It is therefore hardly conceivable that the electroretinogram recorded with standard leads is due to slow potentials in the ganglion cells themselves. Hence it is also excluded that changes in the retinal response due to polarization are determined by effects of the polarizing current on this layer of cells.

7. *Relative latent periods of processes in retina and nerve.* It is difficult to argue in favour of any particular theory of the nature of the retinal electrical response from observations on the latent period in retina and nerve. Nevertheless some facts may be definitely established by this method. For measurements of latencies we used the double cathode ray and two balanced condenser-coupled amplifiers.

It was easy to confirm earlier observations by Adrian and Matthews (1927) and Granit and Therman (1935) to the effect that the negative component P III always precedes the discharge in the nerve. But it is impossible

to know what the positive P II does as its starting point merely shows the moment at which P III is compensated. This regularly occurs later than the first impulses are seen in the nerve. Despite this P II may really precede the nervous discharge.

But at "off" P II rises unimpeded by the simultaneous development of the negative P III. There, however, occurrence of positivity in the retina
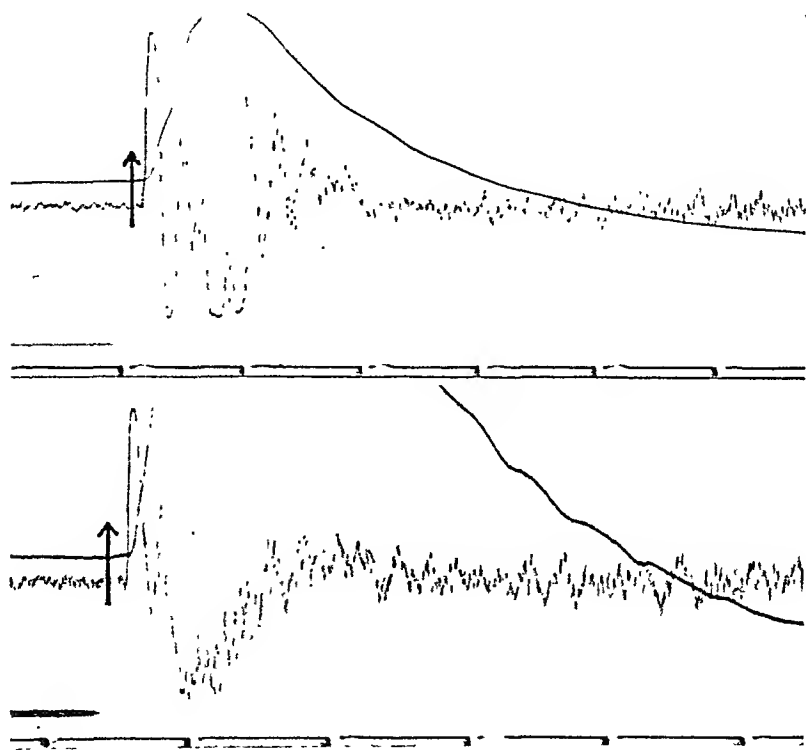
FIG. 6. Off-effects in retina and nerve. Simultaneous records taken with a pair of balanced condenser-coupled amplifiers from retina (upper cathode ray) and nerve (lower cathode ray). Lower record taken with very high amplification of retinal response. Note that retinal response begins with a small slowly rising phase, marked by arrow, before anything happens in the nerve. This phase is hardly visible when lower sensitivity is used, as in upper curve.

before the off-discharge appears in the nerve may indicate return of P III to the base line. As shown by Fig. 6 the appearance of the off-impulses in the nerve is regularly preceded by a small rise of positivity in the retina, but this is visible only at high sensitivity. The steep rising part of the retinal off-effect always follows a couple of milliseconds *after* the nervous discharge had begun. Selective activation of the negative component P III by a flash on top of the off-effect leads to inhibition of the impulses in the optic nerve, followed by a new retinal b-wave accompanied by a fresh discharge in the nerve. The inhibition, as is now well known (Granit and Therman, 1935;

Hartline, 1938), is associated with the characteristic initial a-wave of the retinal response. This wave belongs to the negative component P III (Granit and Riddell, 1934). This is shown in Fig. 7. The impulses stop more abruptly than the a-wave swings down in the retinal record. In the best records a comparison of the time relation of the two events shows that the a-wave in the retina just precedes the onset of inhibition in the nerve. The negativity therefore bears the same relation to inhibition as the small initial positivity to excitation.

DISCUSSION

The two components of the vertebrate retinal response recorded in the standard leads must be localized to the region between the synapses of the ganglion cells and the receptors. The experiments with antidromic impulses would seem to exclude the ganglion cells themselves. It is difficult to imagine both components to be localized to the receptors, and equally difficult to exclude the receptors altogether. Then the retina of *Limulus polyphemus* with only one layer of cells gives one component alone, which with regard to its electrical sign corresponds to the P II of the vertebrate retina (Hartline, 1928; Hartline and Graham, 1932). From the phylogenetic point of view the rods and cones are primitive neurosensory cells (Kappers, Huber and Crosby, 1936), together with the olfactory cells the only ones of this kind carried through the whole progress of evolution. It would therefore seem necessary to identify the retinal response of *Limulus* with one component of the vertebrate electroretinogram. It is possible that the small initial rise of positivity in the retina preceding the off-discharge in the nerve is part of the component P II which is then occluded by the fast return of the negative P III towards the base line (cf. Granit and Therman, 1937).

Recent work by Crescitelli and
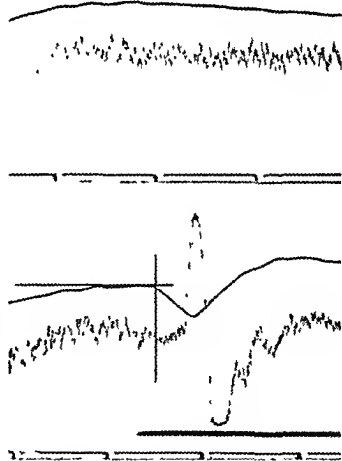
FIG. 7. Re-illumination during off-effect. Simultaneous records from retina (upper cathode ray) and nerve (lower cathode ray). Upper curve, off-effect control; lower curve, re-illumination during off-effect showing negative a-wave of P III in retina and cessation of discharge in nerve.

Jahn (Jahn and Crescitelli, 1939; Crescitelli and Jahn, 1939) shows that the likewise complex retinae of the grasshopper (*Melanoplus differentialis*) and the moth (*Samia cecropia*) are characterized by retinograms in which the characteristic waves of the vertebrate response, the a-, b-, c-, and d-waves, can be identified. However, in the vertebrate retina the corresponding waves are of opposite sign, which probably only is due to the inversion of the latter. A component analysis of these insect retinograms is still lacking. Despite this it is tempting to suggest that one component of the vertebrate retina belongs to the receptorial layer or its synapses, the other one to the synaptic junctions of ganglion cells, amacrines or bipolars, perhaps to all of them. The main fact against placing any component in the receptors has been the evidence for synaptic interaction, demonstrable with the vertebrate retinal electrical response, but the assumption of electrotonic spread offers means of solving this dilemma. It is quite possible that the spread of excitation from the receptors onwards takes place by way of electrotonus rather than by impulses. Our results with polarization show it to be a reasonable proposition to assume that retinal excitability is influenced by electrotonic states.

Somewhat surprising is that "inside cathode," which means that the receptors are lying towards the anode, should cause a change in the direction of increased sensitivity. It reminds one of observations by Erlanger and Blair (1936), according to which anelectrotonus in peripheral nerve supplies a condition which makes it respond repetitively at a temporary cathode. The effects we are dealing with probably depend on ions being deposited on the cells or synapses when the current passes through the intercellular spaces. It is reasonable to assume that the active structures themselves are far less permeable to the galvanic current.

We had hoped that the experiments on polarization would help to solve one of the major problems in retinal electrophysiology: are P II and P III truly opposite potentials or are they merely of opposite sign because the structures generating potential are of opposite orientation relative to the electrodes? Our results make it difficult to understand how the sources generating P II and P III could turn different poles towards the polarizing current and yet be similarly influenced by the latter. It is at least far more reasonable to assume them to be of opposite sign.

SUMMARY

The electroretinogram and the impulses from the optic nerve have been recorded during the passage of a galvanic current across the retina.

"Inside cathode" greatly enhances the discharge in the *optic nerve* and "inside anode" depresses it.

The complex *retinal* electrical response is also enhanced by "inside cathode" and depressed by "inside anode."

After removal of the positive component P II of the electroretinogram

the remaining negative P III reacts to the polarizing current just as the whole response

Antidromic impulses through the optic nerve have no effect whatsoever on the electroretinogram

Simultaneous records of the retinal and optic nerve responses illustrate the practically negligible nerve retinal interval at cessation of illumination

The off-discharge through the nerve is *preceded* by a small initial retinal positivity passing on into the large retinal off-effect the main part of which follows a few milliseconds *after* the discharge in the nerve

A brief discussion of the localization of the components of the retinal electrical response concludes the paper

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THE EFFECT OF ESERINE ON SPINAL REFLEXES IN THE DOG

JEROME K. MERLIS AND HAMPDEN LAWSON

Department of Physiology, University of Louisville School of Medicine, Louisville, Kentucky

CURRENT interest in the action of eserine on the central nervous system derives largely from the acetycholine hypothesis of synaptic transmission. In terms of this hypothesis, eserine, by virtue of its anticholinesterase activity, would be expected to have some effect, probably excitatory, on somatic reflexes.

Reports concerning the central action of eserine have been contradictory, some workers having summarized the action as depression (Rothberger, 1901; Dixon and Ransom, 1924), and others as excitation (Langley and Kato, 1915; Schweitzer and Wright, 1937). The last named authors based their conclusion that eserine is a general excitant for the central nervous system on studies of the effect of the intravenous drug on the knee-jerk of chloralosed cats. In their experiments, however, they observed pure augmentation of the reflex only with the smallest doses, the larger doses producing mixed effects in which depression of the knee-jerk was a prominent feature.

In a preliminary study of the knee-jerk of the dog under barbital anesthesia, the most consistent effect of eserine was a depression of the reflex. We felt justified, therefore, in extending the study to include observations on a more stable reflex, that of the tibialis anticus. In the course of these studies, it was also possible to make some observations on the crossed extension reflex. Although our data are not definitive as regards the central action of eserine, they present the observation that even in the spinal state, flexor and extensor reflexes may be oppositely affected by a drug. It is largely for this reason, and as a criticism of the knee-jerk as a type somatic reflex, that these data are presented.

METHODS

Dogs were anesthetized with either sodium barbital (200–250 mg/kg) or chloralose (80 mg/kg). The knee-jerk was elicited with the solenoid hammer described by Johnson (1927) at intervals of 2, 4, 8, or 16 sec. in the different groups of experiments. At similar intervals the reflex response of the tibialis anticus was elicited by means of an A.C. stimulator of the type described by Bayliss and Eggleton (1935) applied to the posterior tibial nerve through shielded silver wire electrodes. The intensity of the stimulus was adjusted by means of suitable resistances. A rotating key was arranged to close the circuit for hammer or A.C. stimulator for a brief period (0.233 sec.; 14 cycles of the 60 cycle A.C.) at the desired intervals.

Both reflexes were recorded with partially isometric lever systems. In one group of experiments the two reflexes were recorded simultaneously in the same animal, in which case they were elicited in opposite legs, the rotating key being set so as to close the circuits alternately at intervals of 8 sec. In every experiment of this type one or the other circuit was held open from time to time during the observations to make sure that neither reflex

was influencing the other In no case was there evidence of such influence, and the results of these experiments are identical with those in which either reflex was studied alone

Eserine (physostigmine salicylate) was applied locally to the spinal cord by perfusing the lumbar subarachnoid space with Ringer's solution containing the drug in concentrations ranging from 0 025 to 0 2 per cent The solutions were kept within 1–2 degrees of body temperature by means of a constant temperature bath In these experiments the spinal dura was exposed by laminectomy and two No 20 gauge spinal needles were inserted into the subarachnoid space, one at the 11th thoracic segment (inflow) and the other at the 1st sacral segment (outflow) In order to avoid the effects of pressure changes on the spinal cord, a continuous perfusion of Ringer's solution was started at the beginning of an experiment from a reservoir kept under constant pressure from a compressed air line with the aid of a mercury valve A second reservoir at the same temperature and pressure, but containing eserine in Ringer's solution, was connected to the inflow needle through a two way valve, so that instantaneous change of the perfusion fluid was possible The dead space between the valve and the spinal subarachnoid space was 10 cc , which accounts for at least part of the observed latency of the response to subarachnoid perfusion of eserine Perfusion pressure was adjusted to maintain a perfusion rate of 2–4 cc per min , and a record of carotid blood pressure was taken to ensure that perfusion pressure was kept well below systemic blood pressure In confirmation of Luckhardt and Montgomery (1929) it was found that pressure on the spinal cord produced no changes in the reflexes until this pressure attained the level of systemic blood pressure It was attempted, by proper fixation of the needles, to keep the perfusion fluid from coming into contact with extrathecal tissues, but we cannot be sure that this was always completely successful

In another group of experiments, the effects of intravenous eserine were studied The drug was injected in a constant volume of Ringer's solution (5 cc) in doses of 0 01–2 0 mg/kg

RESULTS

The significant observations are summarized in Table 1 As shown by

Table 1 *Intravenous eserine*

Reflex Observed	Number of Dogs	Number of Observations	Effect on Reflex	
			Augmentation	Depression
Knee Jerk				
(Barbital)	9	33	3	30
(Chloralose)	7	27	12	15
Tibialis Anticus				
(Barbital)	16	59	56	3
(Chloralose)	6	34	32	2
Intraspinal eserine				
Knee Jerk				
(Barbital or Chloralose)	8	16	2*	14
Tibialis Anticus				
(Barbital or Chloralose)	7	11	11	0

* Both these observations were made in one dog, non spinal, under sodium barbital anesthesia In this animal, the perfusion of eserine was followed by augmentation, depression, or no change of the reflex, with no apparent relationship between the concentration used and the effect produced

this table, the usual effect of intravenous eserine on the knee-jerk of barbital-ized dogs was depression. Successive injections of the drug were followed by further depression of the knee-jerk (Fig. 1). In no case were we able to demonstrate a reversal of the effect by increasing the dosage of eserine, be-ginning with doses so small as to be ineffective. Under chloralose anesthesia, however, the knee-jerk was augmented nearly as often as it was depressed. Since most of these observations were made on spinal animals (cord tran-sected at T10), the effect of the anesthetic on the response to eserine must have been exerted on the spinal cord itself.
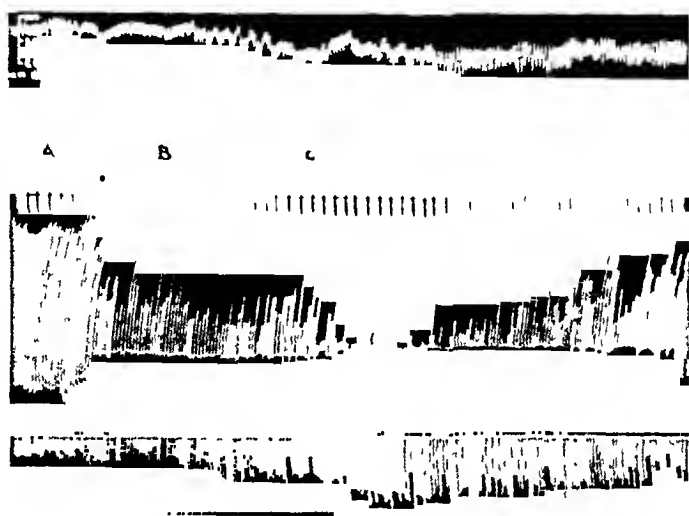
FIG. 1. INTRAVENOUS ESERINE. Dog, 12.3 kg. Sodium barbital, 200 mg/kg. Spinal cord sectioned at T10. Records from above downward are: 1) Carotid blood pressure. 2) Signal. 3) Time, in min. 4) Knee-jerk of left leg. 5) Tibialis anticus reflex of right leg, submaximal stimulus to posterior tibial nerve. At A, B, and C, eserine, 0.5 mg in 5 cc. Ringer's solution injected into jugular vein.

In both chloralosed and barbitalized dogs, intraspinal eserine depressed the knee-jerk in almost all cases (Table 1). In our short series no difference in the effect was apparent with the two anesthetics when eserine was ad-ministered by this route. The intraspinal application of eserine affected the knee-jerk, in most cases, before there was any effect on blood pressure or heart rate (Fig. 2), and in some cases, in the absence of such circulatory changes. The effect on the knee-jerk, therefore, is probably unrelated, in these experiments, to the systemic effects of eserine on the circulation. This is probably true also for intravenous eserine, since it was observed, in con-firmation of Schweitzer and Wright (1937), that atropinization of the animal with reversal of the blood pressure response, did not affect the response of the knee-jerk.

In contrast with the variable, but preponderantly depressant, effect of eserine on the knee-jerk, the tibialis anticus reflex was consistently aug-

mented (Table 1; Fig 1 and 2) This effect was observed equally well under barbital and under chloralose anesthesia, in the spinal and in the intact animal (anesthetized), and with both intravenous and intraspinal eserine Complete atropinization did not abolish this augmentory effect of eserine Large doses of eserine intravenously (1 mg/kg or more) sometimes caused a sustained increase in tone of the tibialis anticus which was accompanied by a diminished amplitude of the reflex response In some of these cases, when tone returned to the normal level, the reflex showed some augmentation above the control level

In some of the acute spinal preparations in which the knee-jerk and the tibialis anticus reflex of opposite legs were alternately elicited, the crossed
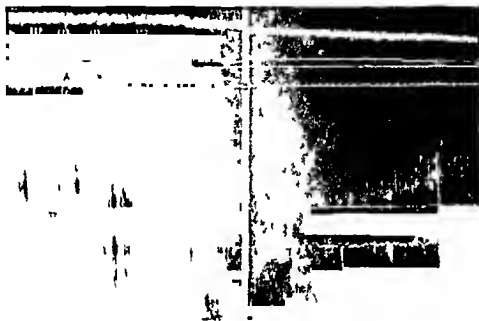
FIG 2 INTRASPINAL ESERINE Dog 22 2 kg Sodium barbital, 200 mg/kg Spinal cord sectioned at T10 Records from above downward are 1) Carotid blood pressure Figures along this tracing are heart rates 2) Perfusion pressure, 3) Signal 4) Time, in min 5) Knee jerk, left leg 6) Tibialis anticus reflex, right leg, submaximal stimulus Between A and B, eserine, 0 1 per cent in Ringer s perfused through spinal subarachnoid space Perfusion rate 3 5 cc per min

extension reflex appeared in sufficient magnitude for observation In these cases, the crossed reflex was augmented along with the flexion response, whereas the knee-jerk in that extremity was depressed In some preparations, the crossed reflex appeared only after eserine was administered, and disappeared again as the other reflexes showed recovery from the effects of the drug

A number of observations have been made on the effect of eserine on the response of the tibialis anticus and the quadriceps to stimulation of their motor nerves (A C stimulator) Simultaneous recording of the reflex response of the muscle of one leg and the response of that muscle in the opposite leg to stimulation of its motor nerve have shown effects on the reflex much earlier, or at lower dosage levels, than on the nerve-muscle response (Fig 3) Even on intravenous administration, only the larger doses of

eserine had a demonstrable augmentory effect on the nerve muscle response. Our observations thus support the conclusion of Schweitzer and Wright (1937) that the effects described cannot be due to an action on the efferent limb of the reflex arc.

After the perfusion of eserine, the dogs often manifested the spontaneous muscular twitches reported by Langley and Kato (1915) to be of central origin. Such spontaneous activity was always confined to myotomes innervated from the perfused cord segments. This is taken as evidence that the action of eserine in these experiments is central rather than reflexly through

FIG. 3. INTRASPINAL ESERINE. Dog, 17.3 kg. Chloralose, 80 mg/kg. Spinal cord sectioned at T10. Records from above downward are: 1) Response of left tibialis anticus to submaximal stimulation of its motor nerve. 2) Response of right tibialis anticus to maximal stimulation of ipselateral posterior tibial nerve. 3) Carotid blood pressure. Figures along this tracing represent heart rates. 4) Perfusion pressure. 5) Signal. 6) Time, in min.

Between A and B, eserine, 0.1 per cent in Ringer's perfused through spinal subarachnoid space. Perfusion rate: 2.0 cc. per minute.

absorption and peripheral action, for in the latter case, segmental localization of the effect would not be observed.

DISCUSSION

The present data exclude the possibility that eserine acts on the efferent limb of the reflex arcs studied. It is considerably more difficult to dismiss the possibility that the drug, by virtue of its widespread visceral action, influences the cord reflexly. The fact that eserine in our study has identical effects on somatic reflexes in the spinal and in the intact animal argues against such a mechanism, since the spinal animal has lost most of its viscerosomatic reflexes. With sufficiently delicate indices to eserine absorption and systemic action, our data on eserine administered intrathecally would be conclusive evidence against this mode of action. Since our data on heart rate and blood pressure are admittedly crude indices, the possibility of absorption, with reflex effects on the cord, cannot be definitely ruled out. Persistence of the action of eserine on the reflexes in the face of full atropinization argues against this possibility. Furthermore, the strictly segmental

distribution of spontaneous muscular twitches following intraspinal eserine points to a localized central, rather than a disseminated peripheral, site of action.

It is tentatively concluded therefore, that the site of action of eserine in these experiments is the spinal centers. If this conclusion be justified, we are led to the important conclusion that this drug selectively augments some, and depresses other, spinal centers. Such selective action through afferent channels, or through effects directly on the higher levels of the neuraxis is not unusual. But we have not been able to find an account of any such action on the spinal cord itself.

The term "antagonistic reflexes" implies that any change in the one reflex will involve a reciprocal change in the other. According to the concept of the Oxford School (Creed et al., 1932) such reciprocity as exists between the knee-jerk and the tibialis anticus reflex is a function of the motoneurone pools of the extensor and flexor muscles. Our data, however, cannot be interpreted entirely on such a basis, for one extensor response, the crossed extension reflex, is augmented coincidentally with the augmentation of the flexion response, whereas the other extensor reflex, the knee-jerk, is depressed. Since both these extensor reflexes are responses of the quadriceps muscle, they are presumably mediated by the same motoneurones. Unless we make the unlikely assumption that the quadriceps motoneurones are fractionated by the two reflexes so that each reflex is mediated by a special group, and that each of these groups is affected differently by eserine, it appears evident that the changes induced in the spinal cord by eserine could not have been confined to the motoneurones.

The conclusion seems inescapable that eserine acts somewhere upstream in the reflex arc, probably on internuncial pathways. On this basis, the depression of the knee-jerk would be regarded as the expression of some sort of inhibitory process taking place in the internuncial pool. Studies on cord potentials (Hughes and Gasser, 1934; Hughes, McCouch and Stewart, 1937) have presented excellent evidence that such inhibition may take place.

Although our knowledge as yet is far too incomplete for full interpretation of the observations, it is permissable to believe that the augmentation of the flexion and the crossed extension reflexes is related to the depression of the knee-jerk. Rather than postulate a selective difference of action of eserine on separate internuncial pools, a reasonable assumption might follow somewhat the lines of Gasser's theory of reciprocal inhibition (Gasser, 1937). According to this theory antagonistic reflexes share some link in the internuncial pathway, so that only one of them may use the common link at any given time. In the terms of this theory eserine might be regarded as having captured the internuncial link for the flexion reflex (and for crossed extension, which uses, in part, the same pathway), thus producing augmentation of these reflexes, and inhibition of the knee-jerk.

Regardless of final interpretation, these observations serve to indicate the inadvisability of characterizing the central action of a drug as excitatory

or depressant from evidence obtained through the study of single reflexes. It is obvious that eserine must be characterized as both excitant and depressant, depending on the reflex chosen. One other point worthy of mention is the discrepancy between the effects observed by us on the knee-jerk of the dog, and those observed by Schweitzer and Wright on the same reflex in the cat. We have no explanation to offer other than the possibility of species differences.

SUMMARY AND CONCLUSIONS

1) Studies have been made of the effect of eserine on the knee-jerk, tibialis anticus reflex, and the crossed quadriceps reflex in chloralosed and in barbitalized dogs.

2) Eserine, administered by perfusion through the lumbar subarachnoid space in the intact or spinal animal (cord transected at T10), depresses the knee-jerk and augments the flexion reflex in almost all cases. The crossed extension reflex is augmented in the extremity in which the knee-jerk is depressed.

3) Eserine given intravenously is predominantly depressant to the knee-jerk, and augmentory to the flexion reflex and the crossed extension reflex.

4) The possible locus of action and the significance of the results are discussed.

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* EEG is used for electroencephalogram, CNS for central nervous system